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CASEIN AND ITS INDUSTRIAL APPLICATIONS

BY
EDWIN SUTERMEISTER

American Chemical Society
Monograph Series

344776

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GENERAL INTRODUCTION

American Chemical Society Series of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic Monographs on chemical subjects. At the same time it was agreed that the National Research Council, in coöperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, Secretary of the American Chemical Society, Washington, D. C.; John E. Teeple, Treasurer of the American Chemical Society, New York City; and Professor Gellert Alleman of Swarthmore College. The Trustees have arranged for the publication of the American Chemical Society series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company of New York City.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed the editors, named at the close of this introduction, to have charge of securing authors, and of considering critically the manuscripts prepared. The editors of each series will endeavor to select topics which are of current interest and authors who are recognized as authorities in their respective fields. The list of monographs thus far secured appears in the publisher's own announcement elsewhere in this volume.

The development of knowledge in all branches of science, and especially in chemistry, has been so rapid during the last fifty years and the fields covered by this development have been so varied that it is difficult for any individual to keep in touch with the progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and such compendia as Beilstein's Handbuch der Organischen Chemie, Richter's Lexikon, Ostwald's Lehrbuch der Allgemeinen Chemie, Abegg's and Gmelin-Kraut's Handbuch der Anorganischen Chemie and the English and French Dictionaries of Chemistry, it often takes a great deal of time to coördinate the knowledge available upon a single topic. Consequently when men who have spent years in the study of important subjects are willing to coördinate their knowledge and present it in concise, readable form, they perform a service of the highest value to their fellow chemists.

It was with a clear recognition of the usefulness of reviews of this character that a Committee of the American Chemical Society recommended the publication of the two series of monographs under the auspices of the Society.

Two rather distinct purposes are to be served by these monographs. The first purpose, whose fulfilment will probably render to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a readable form, intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs will enable such men to form closer contact with the work of chemists in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well digested survey of the progress already made in that field and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, it is intended to include extended references to the literature, which will enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection will be made of those papers which are most important.

The publication of these books marks a distinct departure in the policy of the American Chemical Society inasmuch as it is a serious attempt to found an American chemical literature without primary regard to commercial considerations. The success of the venture will depend in large part upon the measure of coöperation which can be secured in the preparation of books dealing adequately with topics of general interest; it is earnestly hoped, therefore, that every member of the various organizations in the chemical and allied industries will recognize the importance of the enterprise and take sufficient interest to justify it.

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INTRODUCTION

The preparation of this book was undertaken because it was felt that there was no satisfactory work on the subject and that a volume of this sort was therefore needed. It has not proved possible to collect as much information as it was desired to obtain, but it is hoped that the book may still prove of value to those who are interested in the chemistry or commercial applications of casein.

It is a pleasure to acknowledge the splendid co-operation of the contributing authors, without whose generous assistance this work could not have been done.

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Chapter I

The Organic Chemistry of Casein

By Ross Aiken Gortner

Casein belongs to that group of organic compounds known as proteins. The proteins are, without doubt, the most complex organic compounds with which the chemist has to deal, and within this group casein belongs to one of the more complex subdivisions, inasmuch as it is not a simple protein but belongs to the sub-class of phosphoproteins.

Probably no single protein has been the subject of as many chemical investigations as has casein. This is to be expected inasmuch as it is the characteristic protein of the milk of all mammals and can be readily isolated in a more or less pure state. Owing to its ready availability it is but natural that most of the investigations have been conducted upon the casein of cow's milk.

Preparation of casein from cow's milk.—Casein is a protein which has decidedly acid properties and it exists in the milk in the form of a calcium caseinate. The older directions for the preparation of a pure casein simply stated that the casein was precipitated from diluted fat-free milk by acidification. Acetic acid was usually employed for laboratory scale preparations and hydrochloric acid in large scale manufacture. Studies of the physico-chemical constants of casein have recently shown [1] that the isoelectric point of casein is reached at an activity of hydrogen ions corresponding to 2.5×10^{-5} N or in Sörensen's units, a pH of 4.6. Clark, Zoller, Dahlberg, and Weimar [2] (1920) accordingly applied this observation to the manufacture of a casein which could readily be washed free from impurities and which when moist would retain a uniform grain texture and would not pack into a more or less impervious mass. Such conditions are realized by precipitating the casein from skimmed milk which has been heated to 34.5-35° C., by the addition of hydrochloric acid until the mixture attains a pH of 4.8, allowing the curd of casein to settle, draining off the whey and washing the curd repeatedly with a dilute solution of hydrochloric acid in water, this wash solution having a pH of 4.8. Casein so prepared is, after drying, sufficiently pure for technical uses. Further purification may be accomplished by redissolving the moist casein in a dilute alkali, preferably ammonium hydroxide, filter-

ing or centrifuging until a completely clear solution is obtained, and again adjusting the hydrogen ion activity with hydrochloric acid to pH 4.8, followed by repeated washings with water adjusted to a pH of 4.8 with hydrochloric acid. In dissolving the casein in alkali, care must be taken to avoid any considerable excess of alkali, inasmuch as the protein molecule appears to be very sensitive to alkaline treatment.[3,4] The hydrogen ion activity of the alkaline casein solution should never be allowed to exceed pH = 8.5 and preferably should not exceed pH = 7.5.

Following the complete washing of the reprecipitated casein the moist curd is freed as much as possible from water by pressing, and then crumbled into 95 per cent alcohol. After standing for a time, the alcohol is drained off and the casein is completely extracted in a continuous extractor, first with hot alcohol and lastly by ether, followed by drying at a low temperature. The casein should not be dried prior to the alcohol treatment, inasmuch as after drying, the alcohol may fail to penetrate all of the granules.

Casein so prepared forms a snow-white powder which is not appreciably hygroscopic. It has a specific gravity of 1.259. It reacts as a weak acid, is insoluble in water, alcohol and other neutral organic solvents, and readily soluble in water containing the hydroxides or the carbonates of the alkali or alkaline earth metals.

Rakuzin[5] (1915) has investigated the optical rotation of casein solutions. He reports that casein dissolved in dilute (1 : 12) orthophosphoric acid or in acetic acid has a rotation of $(\alpha)_D$ — 86.6°, while if the solvent is a 2 per cent solution of borax or if the casein is dissolved in 2 per cent hydrochloric acid containing 0.2 per cent of pepsin, the values lie between — 93.5° and — 95.4°. Wright (1924) reports $(\alpha)_D$ = — 103° for casein dissolved in N/2 sodium hydroxide.

Casein, like all proteins, is amphoteric and may be dispersed, possibly with true salt formation, in acid solutions of appropriate concentration. Here again we are undoubtedly dependent upon the control of hydrogen ion activity in order to secure reproduceable results. Because of the high hydrogen ion activity of hydrochloric acid, it is much more difficult to completely disperse casein in hydrochloric acid solutions without pH control, than is the case when an acid having a low hydrogen ion activity, such as acetic acid, is used. In any event the hydrogen ion activity should not be allowed to exceed pH 2.5 in order to avoid any possibility of protein denaturization or incipient hydrolysis.

Is casein a homogeneous substance?—Kondo[6] (1925), Linderstrøm-Lang and Kodama[7] (1925), and Linderstrøm-Lang[8] (1925) have conducted an elaborate investigation of the chemical and physico-chemical properties of casein. Linderstrøm-Lang confirms the observation of Kondo that the solubility of casein in dilute solutions of hydrochloric

acid is not a constant value but is dependent upon the amount of undissolved casein which is present. This observation was taken as evidence that casein is not a homogeneous substance but consists of a mixture of two or more proteins which are very similar to each other in chemical composition and which are united in the precipitate in the form of complexes of varying composition and solubility in the same way that Sörensen [9] (1925) suggests that pseudoglobulin and euglobulin are combined in the globulin fraction of the serum proteins of the blood.

Linderstrøm-Lang and Kodama [7] (1925) subjected commercial casein to fractional precipitation and obtained extreme fractions which differed appreciably in their solubility in dilute hydrochloric acid and sodium chloride solutions. Later Linderstrøm-Lang [8] (1925) repeated the fractionation and solubility studies with a sample of casein which he, himself, had prepared in the laboratory, in order to avoid the possibility of the presence of decomposition products which may have been present in the commercial casein. However, he was still able to separate the casein so prepared into fractions which showed decidedly different solubility, and to demonstrate that the solubility was not a fixed value but depended upon the amount of original casein taken for the solubility experiment. The different fractions likewise had different ratios of phosphorus to nitrogen ranging from a maximum of $P/N = 0.0542$ to a minimum of $P/N = 0.0194$. Whether or not the authors are correct in their belief that casein is not a single protein but a mixture of two or more proteins, cannot be decided without further investigation. From the data which they present it would appear that no special precautions had been taken to insure the removal of the alcohol-soluble protein [10] which is always associated with the casein as precipitated from skim-milk. There is a possibility that the alcohol-soluble protein may have fractionated with the casein and have been responsible for the varying solubility and P/N ratios.

Elementary analysis and molecular weight of casein.—Many workers have made elementary analyses of casein from cow's milk, of which those recorded in Table I may be regarded as typical. Table I likewise includes analyses of casein from other species of animals including that of woman's milk. It can readily be seen that caseins prepared from different sources cannot be identified by an elementary analysis.

The determination of the empirical formula is extremely difficult. Cohn, Hendry and Prentiss [17] (1925) have recently reviewed the evidence and conclude that the minimum molecular weight is 12,800 but from other considerations they conclude that the actual molecular weight is probably 192,000. Whether or not they are justified in all of their assumptions is a problem for future research, but the evidence is overwhelming that the actual molecular weight is much greater than 12,800.

TABLE I

REPRESENTATIVE ELEMENTARY ANALYSES OF CASEIN FROM COW'S MILK

	Hammarsten [11] (1883)	Lehman [12] (See Hempel, 1894)	Tangl [13] (1908)	Van Slyke and Bosworth [14] (1913)
Carbon	52.96	54.00	52.69	53.50
Hydrogen	7.05	7.04	6.76	7.13
Nitrogen	15.65	15.60	15.65	15.80
Sulfur	0.72	0.77	0.83	0.72
Phosphorus	0.85	0.85	0.88	0.71

REPRESENTATIVE ELEMENTARY ANALYSES OF CASEIN FROM OTHER SOURCES

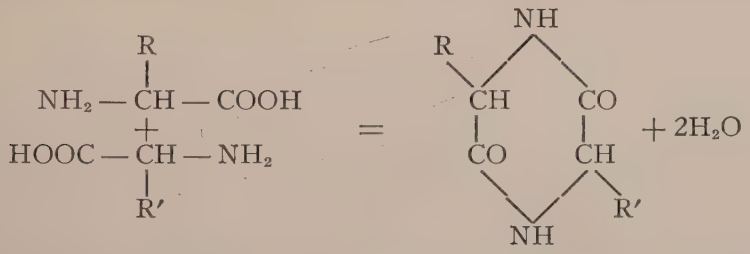
	Woman's Milk (Wroblewski,[15] (1894)	Ewe's Milk (Tangl, 1908)[13]	Goat's Milk (Tangl, 1908)[13]	Mare's Milk (Tangl, 1908)[13]	Ass's Milk (Tangl, 1908)[13]	Rabbit's Milk (Burow, 1905)[16]
Carbon	52.24	52.92	52.90	52.36	52.57	52.87
Hydrogen	7.32	7.05	6.86	7.09	7.01	7.05
Nitrogen	14.97	15.71	15.48	16.44	16.28	15.60
Sulfur	1.12	0.72	0.70	0.53	0.59	0.73
Phosphorus	0.68	0.81	0.76	0.88	1.06	0.81

The amino acid content of casein.—The first decomposition product of a protein which was isolated in pure form was leucine (isobutyl α amino acetic acid) discovered by Proust [18] (1818) in cheese and called by him "oxide caseéux." Glycine (α amino acetic acid) was isolated from gelatin and meat by Braconnot [19] (1820). Liebig [20] (1846) isolated a crystalline compound from casein which had been hydrolyzed by strong alkali. Bopp [21] (1849) and Hinterberger [22] (1849) later isolated the same compound from other proteins using both acid and alkaline hydrolysis and refer to it as tyrosine (p hydroxy benzene α amino propionic acid); Cramer [23] (1865) added serine (β hydroxy α amino propionic acid) isolated from silk gum; Ritthausen [24] (1868) (1869) obtained aspartic acid (α amino succinic acid) and glutamic acid (α amino normal glutaric acid) from vegetable proteins. Weyl [25] (1888) added alanine (α amino propionic acid) obtained from silk fibroin.

These were the only amino acids which were isolated from proteins prior to 1892. During this earlier period the proteins had been studied by many workers using the conventional methods of the organic chemist, oxidation, reduction, destructive distillation, nitration, halogenation, etc., apparently under the assumption that the protein molecule was much less complex than we now know it to be and that the amino acids which had been isolated were secondary decomposition products. Unfortunately most of these studies did not yield much information as to the real nature

of the proteins, and it was not until the epoch-making studies of Emil Fischer [26] (1899-1906 *et seq.*) that an indication of the true nature of the proteins was obtained.

We now know that the amino acids which are obtained from proteins are primary decomposition products and that the proteins themselves may be looked upon as complexes of amino acids condensed together with the elimination of water molecules. The exact mode of such condensation is an open question at the present time. Fischer promulgated the view that the amino acids are united through the peptide linkage, — NH — CO —, a view based largely upon his synthesis of polypeptides and his ability to isolate from natural proteins certain polypeptides identical with those which he had synthesized. This chain-like peptide-linked protein structure has recently been called in question by Abderhalden [27] (1924) who had produced evidence that at least a part of the amino acids in proteins may be present as anhydride combinations in the form of substituted diketopiperazides



and that those diketopiperazide rings are held together by secondary valencies to form the protein molecule. At the present time it is impossible to state how much it will be necessary to modify the peptide linkage structure as formulated by Fischer in order to account for Abderhalden's experimental results. It seems highly probable, however, that both structures exist in the protein molecule.

The amino acid content of casein has been the subject of numerous investigations. Table II shows a compilation of the maximum yields of crystalline amino acids which various investigators have isolated from casein by boiling with mineral acids. The total of 97 per cent shows that the amino acid content of casein is probably better known than is the amino acid content of any other protein of common occurrence or of similar complexity, with the possible exception of zein, the alcohol-soluble protein of *Zea mays,* where the summation slightly exceeds 101 per cent. It should be borne in mind, however, that there is still an appreciable fraction of the casein which is not accounted for, due to the fact that the theoretical recovery should lie somewhere between 110 and 120 per cent of the dry casein. This is due to the fact that the amino

acids are isolated following the hydrolysis of the protein and consequently the weight of the water entering into chemical reaction at the peptide linkages, $R — NH — CO — R' + H_2O = R — NH_2$ and $HOOC\,R'$, causes the amino acids yielded by a protein to weigh considerably more than did the original protein from which they were derived.

The very unsatisfactory state of our methods for the quantitative determination of the amino acid content of proteins is strikingly illustrated by the recent discovery by Dakin [28] (1918) of a new amino acid (β-hydroxy glutamic acid) in casein. This discovery would not have been so surprising were it not for the fact that Dakin was able to isolate the amino acid in an amount equal to 10.5 per cent of the weight of the casein.

Mueller's [29] (1923) later discovery of a new sulfur-containing amino acid in casein only serves to illustrate again the imperfect state of our knowledge regarding the organic chemistry of casein, and of proteins in general.

The diaminotrihydroxydodecanic acid of Fischer and Abderhalden [30] (1904) and the hydroxy tryptophane of Abderhalden and Kempe [31] (1907) have not been isolated from any protein with the exception of casein and their actual existence in casein is extremely doubtful. Abderhalden and Sickel [32] (1924) in a recent study conclude that the compound which has been designated "hydroxy tryptophane" is not a hydroxy tryptophane, and they suggest that it may be β-Bz-oxy-Pr di hydroindolylalanine ($C_{11}H_{14}O_3N_2$). Drechsel [33] (1892) reported the isolation of a diamino acid of the formula $C_2H_6N_2O_2$ from casein, which has not as yet been isolated from any other protein, nor has the isolation been repeated by other workers. Drechsel believed this to be a diamino acetic acid but Willstatter [34] (1902) points out that its properties are not those of the diamino acetic acid which he synthesized. The existence of this amino acid is likewise problematical.

Aside from the amino acids which have actually been isolated from casein there is evidence which indicates the presence of other compounds which have not as yet been isolated. Thus Skraup and Krause [35] (1909) report the presence of 0.85 per cent of methyl groups in the form of methoxy ($— OCH_3$) derivatives and 1.13 per cent of methyl groups attached to nitrogen. Geake and Nierenstein [36] (1914) report 0.88 per cent of total CH_3 groups of which 0.33-0.38 per cent represents $(N)CH_3$ linkages. Herzig and Landsteiner [37] (1914) found 0.64 per cent methoxy-methyl and 1.78 per cent of N-methyl groups and Burn [38] (1914) found 0.77 per cent of $(N)CH_3$ methyl groups. No methoxy or N-methyl compounds have ever been isolated from a protein.

Recently (1925) Dunn [39] has pointed out that there is a liberation

TABLE II

SHOWING THE PERCENTAGES OF AMINO ACIDS *Actually Isolated* FROM CASEIN AS COMPILED FROM ANALYSES OF VARIOUS INVESTIGATORS

Glycine (α amino acetic acid)	0.45 [a]
Alanine (α amino propionic acid)	1.85 [a]
Valine (α isopropyl α amino acetic acid)	7.93 [a]
Leucine (α isopyropyl α amino propionic acid)	7.92 [b]
Iso leucine (β methyl β ethyl α amino propionic acid)	1.43 [b]
Proline (α pyrrolidine carboxylic acid)	8.70 [d]
Phenylalanine (β phenyl α amino propionic acid)	3.88 [a]
Tyrosine (β hydroxyphenyl α amino propionic acid)	5.70 [e]
Glutamic acid (α amino glutaric acid)	21.77 [a]
Aspartic acid (α amino succinic acid)	4.10 [d]
Hydroxy glutamic acid (β hydroxy α amino glutaric acid)	10.50 [d]
Serine (β hydroxy α amino propionic acid)	0.43 [e]
Hydroxy proline (γ hydroxy pyrrolidine α carboxylic acid)	0.23 [e]
Tryptophane (β indol α amino propionic acid)	1.70 [d]
Cystine (di α amino propionic acid ββ′) di sulfide)	0.02 [o]
Arginine (α amino δ guanidine valerianic acid)	4.84 [f p]
Histidine (α amino β imidazole propionic acid)	3.39 [e p]
Lysine (α ε di amino caproic acid)	7.72 [g]
β-Bz-oxy-Pr di hydroindolylalanine ($C_{11}H_{14}O_3N_2$) (possibly)	present [l]
Diaminotrihydroxydodecanic acid ($C_{12}H_{26}N_2O_5$) (existence doubtful)	0.75 [h]
Sulfur-containing amino acid ($C_5H_{11}SNO_2$)	0.40 [k]
? Amino butyric acid ($C_4H_9NO_2$)	present [m]
A diamino acid ($C_2H_6N_2O_2$) (existence doubtful)	present [n]
Ammonia	1.61 [i]
Phosphorus	0.85 [j]
Sulfur	0.76 [j]
Total	96.93

[a] Foreman (1919)[97]
[b] Levene and Van Slyke (1909)[98]
[c] Bissegger (1907)[99]
[d] Dakin (1918)[28]
[e] Fischer (1903)[26b]
[f] Hart (1901)[100]
[g] Van Slyke (1914)[47]
[h] Fischer and Abderhalden (1904)[30]
[i] Osborne and Harris (1903)[101]
[j] Hammarsten (1883)[11]
[k] Mueller (1923)[29]
[l] Abderhalden and Sickel (1924).[32] Originally identified as hydroxy tryptophane by Abderhalden and Kempe (1907).
[m] Foreman (1913)[102]
[n] Drechsel (1892)[33]
[o] Mörner (1901-02).[103] This value is undoubtedly too low. Folin and Looney (1922)[104] by a colorimetric method report 0.25 per cent, and Okuda (1925)[105] by another colorimetric method reports 0.17 per cent.
[p] Osborne, Leavenworth and Brautlecht (1908).[106] These authors report the arginine content as 3.39 per cent and the histidine content at 2.50 per cent. These values are nearer those found by other workers than are the results recorded by Bissegger[99] or Hart.[100]

of carbon dioxide during the acid hydrolysis of a protein. He used casein in his study and the carbon dioxide liberated in a 24 hour hydrolysis corresponded to 0.71 per cent of the weight of the casein; 0.31 per cent was liberated in the first 5 hours of hydrolysis. He suggests that the source of this carbon dioxide may be uramino acids, uramino acid anhydrides (hydantoins) or cyclic diacipiperazines, possibly in such structures as

The nitrogen distribution of casein.—Table III shows the nitrogen distribution of casein from cow's milk as determined by several different investigators using Van Slyke's [40] (1911) method. The ammonia nitrogen is derived chiefly from acid amide linkages, $R-CO-NH_2$, which are present in the protein and which are believed to be associated with the dicarboxylic acids. A small part of the ammonia nitrogen probably originates by deaminization under the influence of the acid used to hydrolyze the protein.[41] The humin nitrogen is in a large measure derived from tryptophane.[42] It will be noted that Van Slyke's nitrogen distribution method gives definite information in the case of only four amino acids, arginine, histidine, lysine and cystine. That the values for arginine, histidine and lysine as reported in Table II are essentially correct is shown by their close agreement, when calculated into terms of percentage of total nitrogen with the figures for these amino acids as given in Table III. Thus 4.84 per cent of arginine is equivalent to 9.86 per cent of the total nitrogen; 3.39 per cent of histidine to 5.81 per cent of the total nitrogen, and 5.95 per cent of lysine to 7.09 per cent of the total nitrogen. The value for cystine as determined by the Van Slyke method under the conditions of hydrolysis employed by Hoffman

TABLE III

THE NITROGEN DISTRIBUTION OF CASEIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

	Van Slyke (1910)[46]	Van Slyke (1914)[47]	Crowther and Raistrick[48] (1916)	Dunn and Lewis[49] (1921)	Hoffman and Gortner[43] (1925)
	Per cent	Per cent	Per cent	Per cent	Per cent
Ammonia nitrogen	10.43	10.27	10.25	10.49	10.20
Humin nitrogen	3.43	1.28	1.20	2.13	1.51
Cystine nitrogen	1.95	0.20	1.24	0.48	1.05
Arginine nitrogen	7.51	7.41	9.22	7.42	9.20
Histidine nitrogen	4.24	6.21	6.82	6.01	6.26
Lysine nitrogen	7.86	10.30	9.62	9.09	8.49
Amino nitrogen in filtrate from bases	55.04	55.81	54.76	58.78	54.12
Non-amino nitrogen in filtrate from bases	9.51	7.13	7.09	5.93	8.76
Total	99.97	98.61	100.19	100.33	99.59

and Gortner [43] (1925) probably represents about 65 per cent of the true cystine nitrogen value.[44] If this be true the cystine (as amino acid) content of casein would be approximately 2.19 per cent. This value is undoubtedly much too high (see footnote "o" attached to Table II). Probably some sulfur compound other than cystine is precipitated by the phosphotungstic acid. It may be that the sulfur-containing amino acid isolated by Mueller [29] (1923) accounts for the greater part of the

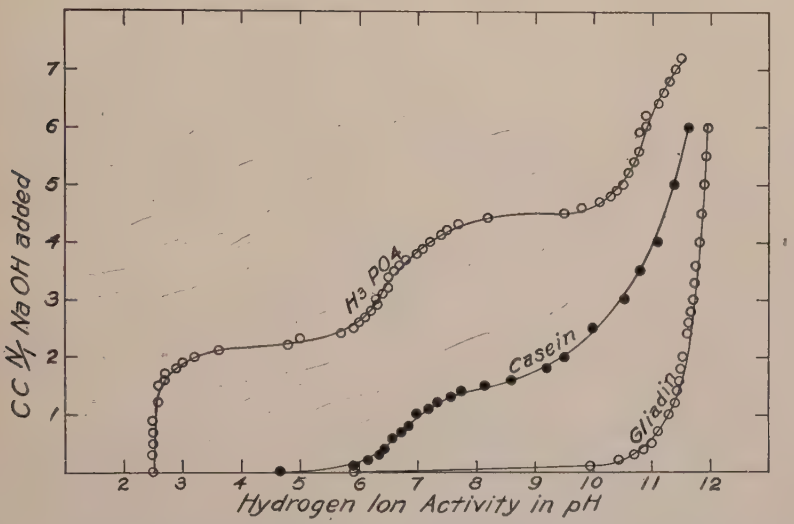


FIG. 1.—The electrometric titration curve of casein as contrasted with similar curves for orthophosphoric acid and gliadin, the alcohol-soluble protein of wheat. (The data from which these curves were drawn are given by Hoffman and Gortner.[43 45])

sulfur in casein, inasmuch as Mueller states that the amount which he was able to isolate is apparently only a fraction of the amount actually present.

The form in which the phosphorus is present in casein is still uncertain although it appears highly probable that it may be present as phosphoric acid. Casein is a relatively strong acid in comparison with other proteins, and its titration curves with sodium hydroxide show a sharp inflection between pH 6.0-7.5. The secondary hydrogen of phosphoric acid is neutralized in this same range of hydrogen ion concentration.[45] Figure 1 shows such a titration curve for casein as contrasted with similar curves for gliadin, the alcohol-soluble protein of wheat, and of orthophosphoric acid.

Are the caseins from the milk of different mammals identical compounds?—As has been noted in connection with Table I, the elementary analyses of the caseins isolated from the milk of different mammals are so nearly identical as to make it impossible to differentiate the sources of the caseins by this method. The physical properties, in so far as they have been studied comparatively, likewise point toward close relationships if not actual identity.

Abderhalden and Schittenhelm [50] (1906) have compared the amino acid content of caseins from cow's and goat's milk. In addition the tyrosine content of woman's milk was compared with that from the cow's and goat's milks. A striking similarity in the composition of the caseins from the different sources was shown and it was evident that variations in the amino acid content of caseins from different mammals could not be used to differentiate the caseins.

The biological reactions of the proteins as developed and practiced by the immunologist have been generally accepted as affording the most delicate and accurate means of identifying the presence or absence of a specific protein or proteins. Such tests with the caseins from different mammals failed to indicate any essential differences between the caseins. Wells [51] (1925) sums up this phase of the question as follows, "An immune serum for any one casein will give reactions with casein from any other species, even of remote relationship," and "Casein from the milk of an animal of any given species shows a closer biologic relationship to the casein of another species than it does to either the whey proteins or to the serum proteins of its own species."

Dudley and Woodman [52] (1915) from a comparative study of the optical activity of the amino acids isolated from racemized casein (*vide infra*) prepared from cow's milk and sheep's milk, came to the conclusion that whereas the amino acid content in the two caseins appeared to be identical, nevertheless there was evidence that the actual arrangement or order of these amino acids as linked in the protein molecule was somewhat different. They accordingly suggest that each species may synthesize its own specific casein but that the differences between the various caseins may be merely the intra-molecular arrangement of the various amino acids.

Paracasein.—Casein is converted into paracasein by the action of the enzyme, rennin. The literature dealing with this phenomenon is very voluminous and the conclusions which have been reached by the various investigators indicate that there is no unanimity of opinion as to the processes which are involved or the reactions which take place.

Van Slyke and Bosworth [14] (1913) prepared casein and paracasein

and made comparative elementary analyses with the following results on the ash-free basis:

	C	H	N	P	S
	Per cent	Per cent	Per cent	Per cent	Per cent
Casein	53.50	7.13	15.80	0.71	0.72
Paracasein	53.50	7.26	15.80	0.71-0.83	0.72-0.87

Geake [53] (1914) reviewed the older literature in so far as comparative analyses of casein and paracasein are concerned and added certain analyses of his own, including elementary analyses and the nitrogen distribution as determined by the Hausmann method, and he reached the conclusion that casein and paracasein are identical in chemical composition, at least in so far as our present chemical methods may serve to differentiate proteins.

Wright [54] (1924) has studied the change in optical rotation of casein and paracasein in the presence of alkali and concludes that the two proteins show the same racemization curves. Wright expresses the belief that the transformation of casein to paracasein is a colloidal phenomenon and that it does not involve changes in the organic chemistry of the casein molecule. Palmer and Richardson [55] (1925) have recently reviewed the more important theories which have been proposed and they conclude that no adequate explanation has as yet been suggested which will account for all of the observed facts. The majority of the theories which have been formulated regard the formation of paracasein as a physico-chemical process rather than a process which involves changes in the chemical composition of the casein molecule. This view agrees with the more recent work. It is difficult to imagine how any deep-seated changes in the organic chemistry of the casein molecule can take place in the extremely short time which is required for the enzyme, rennin, to clot the milk. We can accordingly regard the organic chemistry of paracasein as, in all probability, identical with the organic chemistry of casein.

Racemized casein.—When proteins are treated with dilute alkali and the mixture allowed to stand at room temperature (or preferably at 37° C.) for several days there is a progressive decrease in the optical rotation of the protein solution to a low but practically constant value. When such racemized proteins are hydrolyzed, certain of the amino acids are found to be completely optically inactive, while others have retained their original optical activity. Dakin [56] (1912-13) suggests that the racemization may be due to a tautomeric change of the keto-enol type involving the carbonyl group of the peptide linkage of the protein molecule and the hydrogen on the α carbon atom of the same amino acid.

$$\begin{array}{l} \qquad\qquad\qquad\quad R \\ \qquad\qquad\qquad\quad | \\ -CO-NH-CH-CO-NH-CHR-COOH \longrightarrow \\ -CO-NH-C=C(OH)-NH-CHR-COOH \\ \qquad\qquad\quad | \\ \qquad\qquad\quad R \end{array}$$

Inasmuch as the α carbon atom is asymmetric, the formation of the double bond would destroy its optical activity and when the double bond is later ruptured by acid hydrolysis there would be produced an equal amount of the *d.* and *l.* forms of the amino acid so that the amino acid after isolation would be a racemic mixture, and optically inactive. Dakin suggests that perhaps the explanation as to why certain amino acids in the protein molecule are not racemized may be that "the conditions necessary for racemization of an amino group require the attachment of other groups to both amino and carboxyl radicle." Accordingly those amino acids which occupy terminal positions in the peptide chains of the protein molecule would still retain their optical activity.

Dakin and Dudley [57] (1913) prepared racemic casein by dissolving 500 grams of commercial casein in 5 liters of N/2 sodium hydroxide and incubating the solution for 18-20 days at 37° C. The optical rotation of the solution fell from about —5° to about —3°. The racemic casein was precipitated by acidification with glacial acetic acid and the resulting product, which consisted of a plastic dough-like mass, was repeatedly washed with water until the wash water gave no acid reaction to litmus. After drying for several days at room temperature a light brown friable mass was obtained which could be reduced to a fine white powder, closely resembling the original casein. An elementary analysis gave carbon 53.55 per cent, hydrogen 7.03 per cent, nitrogen 12.5 per cent.

The yield of racemized casein was only about 20 per cent of the original casein which had been dissolved in the alkali. This fact and the fact that considerable ammonia was evolved during the incubation as well as the fact that racemized proteoses remained in the mother liquor following the precipitation of the racemic casein, all point to extensive protein hydrolysis by the action of the alkali as accompanying the tautomeric change responsible for the reduced optical rotation.

Dakin and Dudley [58] (1913) found that racemized casein was not digested by pepsin, trypsin or erepsin when tested *in vitro,* nor was it digested when fed to dogs. Following the feeding, unchanged racemic casein was recovered from the feces. The racemic casein also resisted during a 10-day period the attack of putrefactive bacteria.

The action of halogens on casein.—Numerous preparations have been described as resulting from the action of halogens upon casein. It is extremely doubtful that these represent simple substitution products; more probably partial hydrolysis has simultaneously taken place. The halogen apparently substitutes in the aromatic nuclei of the protein molecule inasmuch as the Millon's reaction (for tyrosine) and the Adamkiewicz' reaction (tryptophane) are invariably negative when the halogenated product is tested.

Chlorine.—Habermann and Ehrenfeld [59] (1901) chlorinated casein by dissolving it in 5 per cent potassium hydroxide, adding potassium chlorate and bubbling hydrogen chloride into the mixture held at room temperature until complete solution took place. The chlorinated product was precipitated by diluting with water and was purified by repeated solution and reprecipitation. Upon drying it became brown and lost its solubility in water. An analysis showed 13.28-14.04 per cent chlorine.

Panzer [60] (1901) prepared a chlorinated product by adding 450 grams of potassium chlorate to a mixture of 1000 grams of casein suspended in 4000 grams of 20 per cent hydrochloric acid. No attempt was made to control the temperature so that the mixture became "very warm." The mixture was allowed to cool and was then strongly diluted with water and the insoluble portion washed with distilled water until free from chlorides. A cream-colored powder weighing 770 grams resulted. This product was insoluble in water or neutral organic solvents, readily soluble in dilute alkalies from which solutions it was reprecipitated by acids. An elementary analysis gave the following figures: C = 47.05 per cent, H = 5.52 per cent, N = 12.40 per cent, S = 0.23 per cent, Cl = 8.32 per cent, P = 0.81 per cent, O = 25.67 per cent.

When this product was hydrolyzed with hydrochloric acid, leucine, glutamic acid, aspartic acid, arginine, histidine, and lysine were identified among the hydrolytic products. In a later paper Panzer [61] (1901-02) described a series of products obtained by the action of sodium ethylate upon the "chlorinated casein." * Apparently he was able to replace a part of the chlorine by the ethoxy group. Salkowski [62] (1923) prepared a "chlorinated casein" by treating 5 grams of casein with 5 grams of sodium chlorate dissolved in 125 cc. of water and adding 20 cc. of 1.126 specific gravity hydrochloric acid. The mixture was heated over a free flame until the liquid had assumed a lemon-yellow color. An excess of sodium hydroxide was added and after cooling, the chlorinated product was precipitated by acidification with hydrochloric acid. The light yellow

* As already noted at the beginning of this section, the methods of preparation of these halogenated products preclude the possibility that they are simple substitution products. The "chlorinated caseins," etc., are undoubtedly chlorinated hydrolytic products of the original casein.

precipitate was filtered and washed, first with water and later with alcohol followed by ether. When dry it formed a fine white powder. Tests for tryptophane and tyrosine were negative. A faint xanthoproteic reaction was obtained indicating that some unchlorinated phenylalanine still remained in the molecule. Analyses gave 6.72-6.78 per cent chlorine and 0.85 per cent phosphorus. The "chlor casein" is appreciably soluble in hot water but is reprecipitated by the addition of sodium chloride to the solution. It is likewise quite soluble in hot 50 per cent alcohol. It is very resistant to the action of pepsin or trypsin *in vitro* and is only slightly attacked by putrefactive bacteria. No indol formation was observed when the "chlor casein" was acted on by indol-producing bacteria.

Bromine.—Hopkins and Pinkus [63] (1898) prepared a number of halogenated proteins. Apparently the only preparation made from casein was the bromine derivative which contained 11.17 per cent bromine. No separate description of its properties or reactions is given. These authors describe their products as having in general the following properties: the xanthoproteic and biuret reactions are positive; the Millon's and Adamkiewicz' reactions are negative as is the reaction for loosely bound sulfur. The halogenated proteins were quite soluble in hot alcohol, difficultly soluble in cold alcohol, water or neutral organic solvents. The halogen derivatives are relatively strong acids, decomposing carbonates and forming stable compounds with the heavy metals, especially lead, mercury, and silver. The products are soluble in dilute alkalis and precipitated by neutralization of these solutions with acetic acid.

Iodine.—Liebrecht [64] (1897) describes several products of the action of iodine on casein. "Periodiocasein" was obtained by stirring an intimate mixture of 80 grams of casein and 20 grams of iodine heated to 100° on a boiling water bath, and later extracting the brown powder with ether until the ether became colorless. The residual ether-insoluble product contained 17.8 per cent iodine. It is a yellow powder soluble in hot dilute alcohol and reprecipitated as brown flocs on cooling. A part of the iodine is loosely bound and can be removed by treatment with sodium thiosulfate.

"Iodiocasein" resulted from the treatment of the "periodiocasein" with sodium thiosulfate. It is a white powder, insoluble in neutral organic solvents and is appreciably acid in reaction, dissolving readily in dilute alkalis and being reprecipitated by acids. It contains both phosphorus and sulfur and according to Liebrecht "can be separated from casein by being insoluble in sodium sulfite solution." "Iodiocasein" contained 5.7 per cent iodine.

A third product, "caseoiodin," containing 8.7 per cent iodine, was prepared by heating 100 grams of "periodiocasein" for two hours with 2000 cc. of 10 per cent sulfuric acid on a water bath. The insoluble

residue was repeatedly dissolved in alkali and reprecipitated with acid followed by a final extraction with hot 70 per cent alcohol. The "caseo-iodin" separated from the alcohol on cooling as colorless flocs which when dried with alcohol and ether formed a white powder.

Desaminocasein.—Skraup and Hoernes [65] (1906), Levites [66] (1904) (1909), Dunn and Lewis [49] (1921), and Lewis and Updegraff [67] (1923) have prepared and studied desaminocasein. This product is prepared by the action of nitrous acid on casein. Dunn and Lewis suspended 100 grams of casein in 2 liters of water and slowly added 140 cc. of glacial acetic acid followed by 500 cc. of an 8 per cent solution of sodium nitrite. Vigorous mechanical agitation was used during the entire process. After standing 18 hours at room temperature the precipitate was filtered on a Buchner funnel and washed repeatedly with hot water followed by a thorough washing with alcohol and later with ether, and dried at 80° C. The yield varied from 90 to 97.5 grams of a fine yellow powder. This product slowly dissolved in 1.5 per cent sodium hydroxide to a red solution. The Hopkins-Cole, Millon's and biuret tests were positive. The nitrogen content was from 0.22 to 0.68 per cent lower than that of the original casein. The free amino nitrogen (derived largely from the $\varepsilon - NH_2$ group of lysine) was greatly reduced, in one sample being entirely absent. Tyrosine is apparently partially destroyed by the action of the nitrous acid. In other respects the protein appears to be but slightly altered. The nitrogen distribution corresponds very closely to that for the original casein when allowance is made for the total absence of lysine due to the removal of its $\varepsilon - NH_2$ group.

The action of proteolytic enzymes on deaminized casein was studied [68] and it was found that this product was no longer attacked *in vitro* directly by erepsin but only after a preliminary digestion with pepsin or trypsin. The digestion by pepsin or trypsin proceeded at a much slower rate than was the case when unaltered casein was used as the substrate. *In vivo* experiments showed that desaminocasein was digested and metabolized in the animal body.

Nitro-casein.—The xanthoproteic reaction of proteins is conducted by treating the protein with concentrated nitric acid, thereby producing a yellow color. It has long been known that this reaction depends upon the presence of aromatic nuclei in the protein molecule. Inouye [69] (1912) nitrated silk fibroin and isolated a nitrotyrosin from the hydrolytic products of this nitro protein. Johnson [70] (1915) later showed that the nitro group was in position 3, *i.e.*, 3 nitro 4 hydroxy phenyl α amino propionic acid. According to both Inouye and Johnson, it is this compound which is responsible for the xanthoproteic reaction.

Von Furth [71] (1899) nitrated casein at room temperature in the presence of urea. The urea was added in order to eliminate the influence

of nitrous acid on the course of the reaction. The *nitro-casein* thus obtained was a bright yellow powder soluble in alkalis to a red brown solution, and precipitated by acetic acid. The Millon's reaction and the lead sulfide reactions were not given by this product.

In the absence of urea during nitration von Furth obtained a second product, *xanthoprotein*. This was apparently very similar to the nitro-casein, but was probably the product of more drastic hydrolysis or decomposition. The ratio of $S : NO_2 : N$ in the xanthoprotein was 1 : 1.5 : 44. Trypsin slowly digested the nitrocasein forming nitrated proteoses and peptones.

Methylated casein.—Skraup and Krause [35] (1909) were apparently the first to methylate casein. Fifty grams of casein was suspended in 100 cc. of absolute alcohol and a solution of 13 grams of potassium hydroxide dissolved in 25 cc. of absolute alcohol was slowly added with constant agitation. The casein dissolved in the alkaline alcohol so that a solution was obtained which was only slightly turbid. To this solution 50 grams of methyl iodide was added and the mixture was heated under a reflux condenser for approximately 2 hours or until the alkaline reaction had disappeared. An additional 50 grams of methyl iodide and 13 grams of potassium hydroxide were then added and the mixture again heated until the alkaline reaction disappeared. The alcohol, together with any unchanged methyl iodide was then distilled off and the residue extracted with 500 cc. of boiling water and the soluble methylated casein purified by repeated precipitation with ammonium sulfate. Following the sixth precipitation with ammonium sulfate exactly enough barium hydroxide was added to remove the sulfate ions as barium sulfate and the barium- and sulfate-free solution was then evaporated to dryness *in vacuo*.

The product so prepared was a faintly yellow powder, reacting slightly acid toward litmus; the biuret and glyoxylic acid reactions were strongly positive but Millon's reaction was negative. Analyses showed the presence of iodine in the final product. Accordingly it cannot be looked upon as a simple methylated protein. The substance analyzed as follows: carbon 52.50 per cent, hydrogen 7.37 per cent, nitrogen 14.22 per cent, sulfur 0.61 per cent, phosphorus 0.87 per cent, iodine 5.35 per cent. The $(O — CH_3)$ determination on several different preparations gave values varying from 1.52-2.07 per cent and an $(N — CH_3)$ determination gave values of 2.79-3.93 per cent.

Geake and Nierenstein [36] (1914) methylated casein using diazomethane as a reagent. Five hundred grams of dry casein were suspended in ether and treated with successive portions of 5 grams of diazomethane in ethereal solution until 75 grams of diazomethane had been used. After each addition of diazomethane the reaction mixture was allowed to stand

in the dark until it became colorless. About four months' time was used in carrying out the methylation. The methylcasein was filtered from the reaction mixture, thoroughly washed with ether and dried. It formed a light white powder, indistinguishable in appearance from casein. It was soluble in dilute alkali (N/50) only after long standing (20 hours) and was even more stable toward dilute acids, being peptized by dilute sulfuric acid only after several days' heating on a water bath. The methyl groups were split off by the alkaline treatment. Analyses gave carbon 55.31 per cent, hydrogen 7.32 per cent, nitrogen 15.49 per cent (Dumas), sulfur 0.800 per cent, and phosphorus 0.755 per cent. Analyses for methoxy and N-methyl groups gave 2.08 per cent $(O)CH_3$ and 2.28 per cent $(N)CH_3$. Determinations of methoxy and N-methyl values for the original untreated casein gave 0.49-0.55 per cent $(O)CH_3$, and 0.87-0.88 per cent $(N)CH_3$, thus methylation increased the methyl groups in casein by 3.48 per cent.

The methyl casein gave all of the usual color reactions characteristic of casein including Millon's reaction which latter would indicate that the p. hydroxy group of tyrosine had not been methylated.

Herzig and Landsteiner [37] (1914) also used diazomethane to methylate casein. They do not give a description of their methylated casein. Methoxy ($O-CH_3$) and N-methyl ($N-CH_3$) determinations on their untreated casein were 0.64 and 1.78 per cent respectively, whereas their methylated casein yielded values ranging from 4.86-5.36 for $O-CH_3$ and 3.85-5.34 for $N-CH_3$ groups.

Edlbacher [72] (1919) later methylated casein by treating a mixture of 1.5 grams of casein and 3 grams of sodium hydroxide in 100 cc. of water with 8 grams of dimethyl sulfate. He does not describe the properties of his methylated casein, inasmuch as he was interested only in the "N-methyl number" which he defines as "that value which indicates the number of methyl groups per hundred nitrogen atoms which are bound to nitrogen by exhaustive treatment with dimethyl sulfate in alkaline solution." He finds an N-methyl number of 19.7 for methylated casein.

Vaughan's "protein poison" from casein.—Vaughan [73] (1913) has shown that when proteins are heated with a 2 per cent solution of sodium hydroxide in absolute alcohol, the protein molecule is hydrolyzed and can be separated into at least two fractions, one which is insoluble in absolute alcohol and is physiologically inert, and the other soluble in absolute alcohol and possessing extremely poisonous properties when injected intravenously, intra-abdominally, or subcutaneously into experimental animals, the symptoms of death being very similar to those of anaphylactic intoxication.

Leach [73] (Vaughan, 1913), Underhill and Hendrix [74] (1915), and Underhill and Hjort [75] (1922), as well as the Vaughans [73] (1913) have

prepared this "protein poison" from casein. The casein, together with 15 to 25 times its weight of absolute alcohol containing 2 per cent sodium hydroxide, was placed in a flask fitted with a reflux condenser, and the mixture was heated on a boiling water bath for one hour. The liquid was then decanted from the insoluble residue, and the residue extracted twice more in a similar manner. The liquids were then united, filtered and carefully neutralized with hydrochloric acid. After filtering from the precipitated sodium chloride, the alcoholic solution of the toxin was evaporated to dryness *in vacuo* at 40°. The resulting product was a dark brown scale which powdered to a light brown highly hygroscopic powder. No analytical data in regard to composition are given. This powder was readily soluble in water to an acid, slightly opalescent solution. The toxin diffuses very slowly through collodion when dialyzed against distilled water. The biuret, Millon's, Adamkiewicz', and xanthoproteic reactions are strongly positive. Precipitates are produced by such protein precipitants as phosphotungstic acid, phosphomolybdic acid, uranyl acetate, picric acid, mercuric chloride, platinic chloride, lead acetate, etc.

Underhill and Hendrix [74] (1915), Ringer and Underhill [76] (1921), Underhill, Greenberg and Alu [77] (1921), Underhill and Hjort [75] (1922), and Underhill and Ringer [78] (1922) have made a series of studies on the physiological action of Vaughan's crude soluble poison. Their results indicate that the poison possesses a greater toxicity than is shown by the ordinary proteoses and peptones. "The fatal outcome following the injection of the poison occurs in two ways, (1) a primary, due to respiratory failure within two hours following its administration, and (2) a secondary, occurring in 2 to 36 hours as the result of a progressive asthenia." [75] The intravenous injection produced a prolonged fall in arterial blood pressure, a delay in blood clotting time (normal clotting time of seven minutes, whereas when the poison had been injected the blood was not clotted in 24 hours) and a marked increase in blood concentration. The reader must be referred to the original papers for details of the physiological studies.

A careful study of the soluble protein from egg albumen [73] shows conclusively that the crude protein poison prepared as noted above, must be considered as a mixture from which a number of non-poisonous fractions can be separated. The toxin was finally concentrated so that 0.5 mgr. when injected intravenously was sufficient to kill a guinea pig of 200-300 grams weight. The exact chemical nature of the poison is still unknown.

Formaldehyde casein.—Formaldehyde reacts with proteins to form more or less stable compounds in which the original properties of the protein are profoundly altered. Such proteins have been prepared by Trillat [79] (1892), Blum [79a] (1896), Benedicenti [80] (1897), Schwarz [81]

(1900) and Treves and Salomone [82] (1908). Blum suggests that the reaction involved is a condensation of the free amino groups with the aldehyde with the corresponding elimination of water.

$$\begin{matrix} -NH_2 \\ \\ -NH_2 \end{matrix} + CH_2O = \begin{matrix} -NH \\ > CH_2 + H_2O \\ -NH \end{matrix}$$

or

$$-NH_2 + CH_2O = -N = CH_2 + H_2O$$

Benedicenti [80] (1897) suggests in addition to these linkages the possibility of an aldehyde-ammonia type of reaction:

$$R-NH_2 + HCHO = R-NH-C\begin{matrix} OH \\ \diagup \\ -H \\ \diagdown \\ H \end{matrix}$$

Incidentally he remarks that it is idle to speculate in regard to what linkages may be involved until we know a great deal more about the actual chemical configuration of the protein molecule.

Benedicenti [80] (1897) prepared formaldehyde casein by suspending casein in five times its weight of water and adding 4 cc. of 2 per cent formaldehyde solution for each 5 grams of casein. The mixture was then allowed to stand at room temperature. At the end of 16 days, analyses showed that each gram of casein had combined with 0.0057 gram of formaldehyde. The resulting product was insoluble in water, did not swell (become hydrated) when treated with dilute hydrochloric acid, nor was it soluble in the dilute acid, and was not attacked by pepsin, trypsin, rennet, or bacteria. When the formaldehyde casein was suspended in water and the mixture subjected to steam distillation until 200 grams of distillate had been collected from 5 grams of aldehyde casein, practically all of the "bound" formaldehyde was found to be in the distillate. The residue in the flask showed in certain tests the properties of unaltered casein, *i.e.*, it readily swelled (hydrated) in dilute hydrochloric acid and was readily digested by proteolytic enzymes. The only point which was noted in which it differed from normal casein was that it gave a red biuret reaction instead of the normal violet. This apparently indicates partial hydrolysis of the peptide linkages, probably caused by the process of steam distillation. The fact that formaldehyde can apparently be readily removed from formaldehyde-protein complexes is at least suggestive that the aldehyde-ammonia reaction involving the acid amide groups may be the principal linkage of formaldehyde and proteins.

The chemistry of casein and formaldehyde in the manufacture of plastic masses which harden to resemble bone or ivory will be treated in a later chapter.

"Oxyprotosulfonic acid."—Bondyznski and Zoja [83] (1894) oxidized casein with potassium permanganate (15 grams of permanganate in 1 liter of water for each 25 grams of protein) by allowing the mixture to stand at room temperature for 10 days. Inasmuch as the manganous oxide had not settled so that a clear filtrate could be obtained, alkali was added. The manganous oxide separated at once, leaving a clear supernatant liquid which was filtered off and acidified with hydrochloric acid, causing a precipitation of the "oxyprotosulfonic acid." This was filtered off, dissolved in dilute alkali and reprecipitated with acid. After washing to the absence of chlorides, it was dried, first at room temperature and later at 110°. The following elementary analysis is typical of their product: C = 52.07 per cent, H = 6.81 per cent, N = 14.91 per cent, S = 0.76 per cent, P = 0.70 per cent.

Buraczewski and Krauze [84] (1911) were able by the use of glacial acetic acid as a solvent to fractionate this product, showing that it was not a simple compound but a mixture.

Further investigations are reported by Schuberthowna [85] (1912) who separated the original product into seven distinct fractions on the basis of differential solubilities in organic solvents. From the data available it is evident that oxidation of casein with potassium permanganate produces not only oxidation but a simultaneous hydrolysis of the protein molecule and that "oxyprotosulfonic acid" is simply a name given to a heterogeneous mixture which can be obtained from a protein when certain specific laboratory processes are carried out.

Caseinkyrine.—Siegfried [86] (1904) (1906-07) isolated a basic substance from casein which had been partly hydrolyzed by being treated with ten times its weight of 12-16 per cent hydrochloric acid for three weeks at a temperature of 38-39° C. The product formed a crystalline precipitate with phosphotungstic acid but the free "kyrine sulfate" could not be obtained in crystalline form. An analysis of the sulfate indicated its formula to be $C_{23}H_{47}N_9O_8 . 3H_2SO_4$. The amino acid content corresponded to one molecule of arginine, two of lysine, and one of glutamic acid. All three of these amino acids were isolated after complete hydrolysis of the kyrine.

Levene and van der Scheer [87] (1915) in a repetition of Siegfried's work * were able to separate the kyrine fraction into two polypeptides, one of which gave a crystalline sulfate. The one forming the crystalline sulfate was apparently a tripeptide composed of lysine, oxyproline, and

* Unfortunately their experimental procedure was somewhat different. They hydrolyzed at 40° C. with 12.5 per cent hydrochloric acid for five weeks.

valine, although lysine was the only amino acid which they succeeded in isolating in any appreciable quantity.

Skraup and Witt [88] (1906) repeated Siegfried's work and were unable to agree with him that casein contained a kyrine nucleus. They found that the "kyrine" phosphotungstate could be fractionated either in water solution or in alcohol solutions of various concentrations into a number of fractions showing different solubilities and showing different chemical properties. When the "kyrine" fraction was converted into a picrate, they were able to isolate an appreciable amount of free lysine picrate.

Paranuclein and paranucleic acids from casein.—Hammarsten [11] (1883) digested casein *in vitro* with pepsin and found that a phosphorus-rich precipitate separated during the process. This precipitate he believed to be a nuclein. He describes it as insoluble in water or in dilute (0.2 per cent) hydrochloric acid, or in dilute acetic acid. He states that the nuclein is present in the casein as an integral part of the casein molecule and is split off during the process of peptic digestion.

Malfatti [89] (1892) later prepared the same product by the peptic digestion of casein and found that it dissolved readily in ammoniacal solutions. He was, however, unable to isolate any purine bases from the substance after hydrolyzing with dilute sulfuric acid. He accordingly suggests that in order to prevent confusion with the true nucleins which do contain purine bases, this compound and other compounds of a similar nature should be called *paranucleins* or *para nucleic acids*.

Plimmer and Bayliss [90] (1905-06) studied the action of pepsin, trypsin, and papain on casein, with especial reference to the fate of the casein phosphorus. They found that trypsin converted practically all of the phosphorus into a soluble form (*i.e.*, not precipitable by tannin) within 24 hours and that the "soluble P_2O_5" consisted of approximately 35 per cent of inorganic phosphoric acid and 65 per cent of organic phosphorus compounds. Pepsin on the other hand even after a 146 day digestion had converted only about 70 per cent of the phosphorus into a "soluble form" and even then the soluble phosphorus was almost wholly in organic combination. The action of papain was intermediate between that of trypsin and pepsin.

Salkowski [91] (1896) (1899) (1901), Salkowski and Hahn [92] (1895), Alexander [93] (1898), Reh [94] (1908), Dietrich [95] (1909) and others have made investigations of the phosphorus-containing compounds which can be isolated from the peptic digests of casein.

Salkowski's preparation which he called *para nucleic acid* was soluble in water, forming a solution sufficiently acid to decompose carbonates of the alkalis or alkaline earths, insoluble in alcohol and acetic acid, was levo-rotatory $\alpha_D = -46°$, aqueous solution formed precipitates with an

aqueous solution of egg albumen, lead acetate, copper acetate, tannin, and phosphotungstic acid in hydrochloric acid solution, etc. An analysis gave: C = 42.51 per cent, H = 6.97 per cent, N = 13.25 per cent, and P = 4.31 per cent.

Reh's product was prepared by precipitation with uranyl acetate and contained eight times the phosphorus content of casein (6.9 per cent : 0.83 per cent P). After hydrolysis of 380 grams of the uranyl precipitate containing approximately 200 grams of organic material, Reh separated glutamic acid (13.4 gr.), aspartic acid (1.0 gr.), lysine (7.0 gr.), isoleucine (1.5 gr.), amino valerianic acid (1.4 gr.), leucine (1.3 gr.), proline (0.9 gr.), histidine (0.4 gr.), phenylalanine (0.1 gr.), alanine (0.1 gr.). Tyrosine was present as indicated by Millon's reaction. Reh suggests that in all probability his preparation represents a mixture of two or more acids having the structure of a large polypeptide molecule united to one of the hydrogens of phosphoric acid.

Dietrich [95] (1909) confirmed this belief by fractionating the uranium precipitate with zinc, copper, and lead salts. He obtained at least four different products containing 10.0, 4.1, 3.84, and 3.88 per cent of phosphorus respectively. The corresponding N : P ratios were 1 : 1, 3.07 : 1, 2.59 : 1, and 3.87 : 1. The product having the N : P ratio of 1 : 1 was not a simple amino acid phosphate as the ratio might suggest but contained at least proline, glutamic acid, and lysine. The amount of these three amino acids which was separated did not account for all of the molecule so that in all probability other amino acids were present.

In view of the diverse results noted above, it is difficult to draw a conclusion as to whether anyone has actually isolated from casein a true "nuclein" or a "para nuclein" in the sense of a specific phosphoric acid-containing nucleus of the original casein. It would appear probable that the products actually isolated represent mixtures of various phosphorus-containing peptones and peptides and that the divergent opinions and analyses recorded in the literature are due to different experimental conditions, including the different proteolytic activity of the pepsin preparations, prevailing in the different laboratories. The problem of the nature of the phosphorus linkages in casein still remains to be elucidated.

The action of carbon disulfide on casein.—Uhl [96] (1913) treated 5 grams of casein dissolved in 100 cc. of 5 per cent sodium hydroxide with 2 cc. of carbon disulfide and allowed the mixture to stand with frequent shaking for several days in a closed container. The mixture was then boiled under a reflux condenser for several hours, following which treatment the excess carbon disulfide was allowed to distil off. He thus obtained a clear golden yellow solution. The reaction involved is stated to be

$$R-NH_2 + CS_2 = R-NH-\overset{\overset{S}{|}}{C}-SH.$$

The sulfur compound was isolated by adding an ammoniacal solution of copper acetate (4 grams) and precipitating the mixture with acetone. The copper salt so formed was an amorphous brown powder containing 17.91 per cent copper and was readily soluble in faintly alkaline solutions. No other properties of the casein preparation are given.

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Chapter 2

Physical Chemistry of Casein

By John Arthur Wilson

The casein molecule, like other protein molecules, may be looked upon as built up of many amino acids, the acid group of one being in combination with the basic group of the next, forming long chains and cross chains in the relatively huge molecule of protein. When a mass of the protein is brought into contact with water, it first swells by absorbing water and it appears probable that the water molecules diffuse in between the chains of amino acids making up the protein molecules. Even though the protein itself may not be in solution, the amino acid groups now wet by water are capable of reacting with materials in the water just as though they were themselves dissolved, but restricted in their movement.

In such systems, it is often extremely difficult to make quantitative studies because of lack of means for isolating the several phases. Failure to recognize the existence of all of the phases present and to measure hydrogen-ion concentrations has made it difficult to interpret or to use much of the vast amount of published data on casein. It is only within the past few years that many of the fundamental principles of the physical chemistry of casein have come to be recognized. In this chapter only the modern work will be discussed.

Isoelectric Point

Amino acids are amphoteric electrolytes, functioning and dissociating both as acid and as base. This property is not lost when they are joined into a protein molecule and the protein itself dissociates as both acid and base. In contact with solutions of hydrochloric acid, for example, the amino groups combine with HCl, yielding a highly ionizable protein chloride. In a similar manner, the acid groups of the protein react with NaOH to produce a highly ionizable sodium proteinate. In the acid solution the protein structure is cationic, but its electrical charge becomes decreasingly positive upon the addition of alkali, passing through a neutral point, and then becoming increasingly negative. The point of

electrical neutrality is called the isoelectric point and it is the point where the protein functions equally as cation and anion.

It is customary to define the isoelectric point of a protein as the pH value of the solution producing electrical neutrality in the protein structure. Michaelis and Pechstein [1] found the direction of migration of casein in an electrical field to change in a zone limited by the pH values 4.9 and 4.3 and that its point of minimum solubility occurs at pH = 4.6, from which it is evident that the isoelectric point of casein is 4.6.

Solubility

As a rule, protein ions are much more soluble than undissociated protein. Cohn found that the solubility of proteins in the neighborhood of their isoelectric points is generally greater the more they are dis-

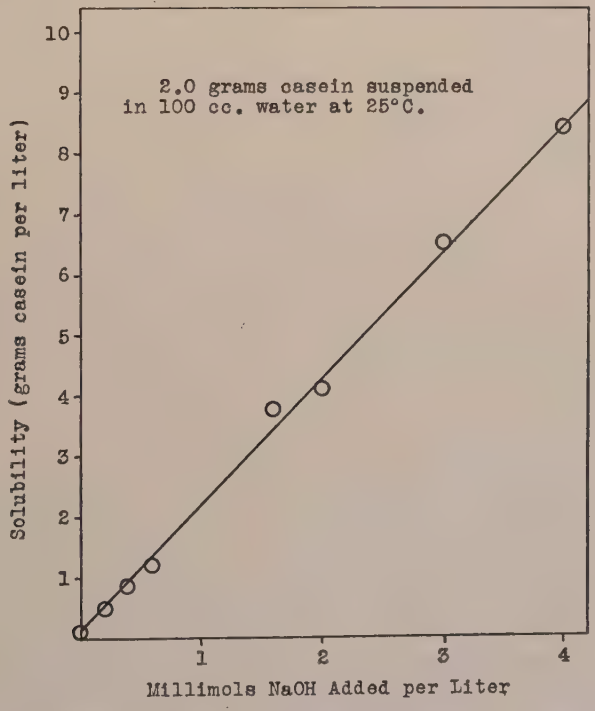

FIG. 1.—Solubility of casein as a function of amount of added alkali.

sociated.[2] He also measured the solubility of casein at its isoelectric point and found it to be 0.11 gram per liter at 25° C.[3] This value includes both dissociated and undissociated casein.

In a study of the effect of adding sodium hydroxide to suspensions of casein in water, Cohn and Hendry [4] found the solubility of casein to increase in direct proportion to the amount of NaOH added, as shown in Figure 1. They also found that casein forms a well defined disodium compound. The dissociation of casein as a divalent acid may be expressed as follows:

$$[H^+] \times [HCas'] = K_1 \times [H\ Cas]$$
$$[H^+] \times [Cas''] = K_2 \times [HCas']$$

These may be combined thus

$$[H^+]^2 \times [Cas''] = K_1 \times K_2 \times [H_2Cas]$$

Taking concentrations in grams per liter, the solubility product constant $[H^+]^2 \times [Cas'']$ was found to be 2.2×10^{-12} at 25° C. The solubility of undissociated casein $[H_2Cas]$ is 0.09 gram per liter, from which the product of the acid dissociation constants $K_1 \times K_2$ may be calculated as 24×10^{-12}. Casein is thus a stronger acid than carbonic and will liberate carbon dioxide from solutions of carbonates and bicarbonates. Cohn [5] gives the equivalent weight of casein as an acid as from 2,096 to 2,166 and the probable molecular weight as 192,000.

In dealing with the solubility of casein in hydrochloric acid solution, we are confronted with a mechanism of solution distinctly different from that involved in the solution of casein in alkaline solutions. Sodium caseinate passes into solution in a manner analogous to the dissolving of sodium oleate, but casein in acid solution dissolves only after it has first swollen beyond a certain limit. A consideration of this swelling involves the principles of the Donnan equilibrium.

The Donnan Equilibrium

In dispersions of casein salts important complications result from the fact that the casein ions are practically non-diffusible whereas the oppositely charged inorganic ions are diffusible. Donnan [6] propounded a theory, based upon the well known distribution law, to account for the type of equilibrium resulting from the separation by a membrane of two solutions, one of which contains an ionogen having one ion that cannot diffuse through the membrane, which is permeable to all other ions of the system. His theory was later developed to explain the peculiar behavior of protein jellies by Procter and Wilson [7] and the behavior of colloidal dispersions in general by Wilson.[8] The soundness of the theory has been firmly established by the extensive researches of Jacques Loeb [9] and others.

As an example, Donnan takes an aqueous solution of a salt, NaR,

such as Congo red, in contact with a membrane which is impermeable to the anion R′ and the non-ionized salt NaR, but will allow water and Na^+, or any other ion, to pass freely through it. The membrane separates the Congo red solution from an aqueous solution of sodium chloride, which will diffuse from its Solution II into the Solution I of NaR.

The problem of determining how the sodium chloride will distribute itself between the two solutions on either side of the membrane can be solved by the following considerations. When equilibrium has been established, if a small virtual change is made reversibly at constant temperature and volume, the free energy will remain unchanged, that is, no work will be done. The change here considered is the transfer of *dn* mols of Na^+ and Cl′ from II to I. The work, which equals zero, is

$$dn.RT.\log_e\frac{[Na^+]_{II}}{[Na^+]_I} + dn.RT.\log_e\frac{[Cl']_{II}}{[Cl']_I} = 0$$

whence

$$[Na^+]_{II} \times [Cl']_{II} = [Na^+]_I \times [Cl']_I$$

The brackets indicate concentration in mols per liter. Equilibrium will be established only when the product of the concentrations of Na^+ and Cl′ has the same value on both sides of the membrane.

Since this equation of products is of vital importance to the quantitative development of the theory here presented, any doubt as to its validity should be dispelled at the outset. The derivation of the equation need not involve the use of thermodynamics, because it can readily be visualized. In passing from one phase to the other, the oppositely charged ions must move in pairs, since they would otherwise set up powerful electrostatic forces that would prevent their free diffusion. For this reason, a sodium or a chlorine ion striking the membrane alone could not pass through it. But, since the membrane is freely permeable to both Na^+ and Cl′, when two oppositely charged ions strike the membrane together, there is nothing to prevent them from passing through into the solution on the opposite side. The rate of transfer of these ions from one solution to the other depends, therefore, upon the frequency with which they chance to strike the membrane in pairs, which is measured by the product of their concentrations. At equilibrium, the rate of transfer of Na^+ and Cl′ from Solution II to Solution I exactly equals the rate of transfer of these ions from Solution I to Solution II, from which it follows that the product of the concentrations of these ions has the same value in both solutions.

We may now consider the effect of adding another salt, such as KBr, to the system. Following the same line of reasoning, it will be evident that equilibrium will be established only when the product $[K^+] \times [Br']$

has the same value on both sides of the membrane and the same will be true for the products $[K^+] \times [Cl']$ and $[Na^+] \times [Br']$. In fact, with any number of monomonovalent ionogens present in the system, the product of the concentrations of any pair of diffusible and oppositely charged ions will have the same value in both solutions.

Introducing polyvalent ions into the system makes the equation of products but very little more complicated. When a polyvalent ion strikes the membrane, it will pass through only when an equivalent number of ions of opposite sign strike the membrane at the same time and pass through with it. The rate of transfer of any dissociated ionogen from one solution to the other, across the membrane, is evidently determined by the product of the concentrations of all of the ions required to produce the undissociated ionogen. At equilibrium, this product will have the same value in both solutions. If, for example, the system contained the ions Na^+ and SO_4'', then the product $[Na^+] \times [Na^+] \times [SO_4'']$, or $[Na^+]^2 \times [SO_4'']$, would have the same value on both sides of the membrane, at equilibrium.

With the equation of products as a starting point, we may now consider further the nature of the unequal distribution of ions between the two solutions, caused by the impermeability of the membrane to the anion R′. In Solution II of the simple system including only the ionogens NaR and NaCl, let

$$x = [Na^+] = [Cl'].$$

In Solution I, let

$$y = [Cl']$$

and

$$z = [R']$$

whereupon

$$[Na^+] = y + z.$$

The equation of products may now be written

$$x^2 = y(y + z).$$

But here we have the product of equals equated to the product of unequals, from which it is apparent, mathematically, that the sum of the unequals is greater than the sum of the equals, or that

$$2y + z > 2x.$$

The reasoning thus indicates that the concentration of diffusible ions in Solution I, at equilibrium, is greater than in Solution II. If we let the excess of concentration of diffusible ions of Solution I over Solution II be represented by e, then

$$2y + z = 2x + e$$

and

$$x = y + \sqrt{ey}$$

which shows us further that x is greater than y, or that the concentration of ionized sodium chloride is greater in Solution II than in Solution I. In spite of the fact that the membrane is completely permeable to sodium chloride, this salt does not distribute itself equally throughout both solutions, but is the more concentrated in Solution II, at equilibrium.

This different distribution of ions in the solutions at equilibrium gives rise, not only to a difference in osmotic pressure, but also to an electrical difference of potential across the membrane. Donnan derived the equation for this potential difference by the following thermodynamic reasoning.

In the system just described, let π_I be the potential for positive electricity of Solution I and π_{II} that for Solution II. Let the extremely small quantity Fdn of positive electricity be transferred isothermally from II to I. In this virtual change of the system from equilibrium, the following work terms must be considered: The change in free electrical energy represented by $Fdn(\pi_I - \pi_{II})$ and the simultaneous transfer of pdn mols of Na^+ from II to I and of qdn mols of Cl' from I to II, where p and q are the respective transport numbers of the ions, and hence $p + q = 1$. The maximum osmotic work of operation of this transfer of ions is represented by the expression

$$pdn.RT.\log_e\frac{[Na^+]_{II}}{[Na^+]_I} + qdn.RT.\log_e\frac{[Cl']_I}{[Cl']_{II}}$$

But, since the system is in equilibrium, the electrical virtual work must balance the osmotic virtual work, or

$$Fdn(\pi_I - \pi_{II}) = pdn.RT.\log_e\frac{[Na^+]_{II}}{[Na^+]_I} + qdn.RT.\log_e\frac{[Cl']_I}{[Cl']_{II}}.$$

But $\dfrac{[Na^+]_{II}}{[Na^+]_I} = \dfrac{[Cl']_I}{[Cl']_{II}} = \dfrac{x}{y}$ and $p + q = 1$. Letting $E = \pi_I - \pi_{II}$, we have

$$E = \frac{RT}{F}\log_e\frac{x}{y} \text{ volts.}$$

This is an equation of fundamental importance in dealing quantitatively with the electrical phenomena associated with colloidal behavior.

It will now be shown that this equation is still valid when other ions of any valency are added to the system. Consider the general case where

an ionogen yielding the ion M^{a+} of valency a is added. By applying the above line of reasoning to the potential difference produced by the unequal distribution of the ions of the added ionogen between Solution I and Solution II, we arrive at the equation

$$E = \frac{RT}{nF} .\log_e \frac{[M^{a+}]_{II}}{[M^{a+}]_{I}}$$

where $n = a$, the valency of M^{a+}. But it is evident from the equation of the products that

$$[M^{a+}]_I \times [Cl']^a_I = [M^{a+}]_{II} \times [Cl']^a_{II}$$

and that

$$[Na^+]^a_I \times [Cl']^a_I = [Na^+]^a_{II} \times [Cl']^a_{II}$$

from which it is apparent that

$$\frac{[M^{a+}]_{II}}{[M^{a+}]_{I}} = \frac{[Na^+]^a_{II}}{[Na^+]^a_{I}} = \frac{x^a}{y^a}$$

Therefore,

$$E = \frac{RT}{aF} .\log_e \frac{x^a}{y^a} = \frac{RT}{F} .\log_e \frac{x}{y}.$$

This equation shows that the unequal distribution of the added ionogen between Solution I and Solution II at equilibrium is producing exactly the same potential difference as the unequal distribution of sodium chloride. Although the addition of any ionogen must produce a change in the measured potential difference, by disturbing the equilibrium, all ionogens present when equilibrium is again established are producing the same potential difference, regardless of valency. The potential difference can thus be calculated from the measurement of distribution of only one kind of ion between the two solutions.

The complexity of the systems just described is due to the presence of the non-diffusible ion R′ in one of the phases. In all aqueous dispersions of casein, the protein ions are practically non-diffusible and this gives rise to the conditions involved in the Donnan equilibrium.

Swelling and Solution in HCl

When purified casein is brought into contact with a dilute solution of HCl, it swells by absorbing some of the solution and a transparent jelly results. The acid diffuses into the jelly and combines with casein, forming a highly ionizable casein chloride. When equilibrium has been established, the solution absorbed by the jelly bears a relation

to the outer solution analogous to the relation existing between solutions I and II described in connection with the Donnan equilibrium. The outer solution contains H^+ and Cl' in equivalent concentrations, while the jelly contains ionized casein chloride in addition to ionized HCl, making the concentration of Cl' greater than that of H^+.

In the outer solution let

$$x = [H^+] = [Cl'].$$

In the jelly let

$$y = [H^+]$$

and

$$z = [Cl'] \text{ from ionized casein chloride.}$$

Then

$$\text{total } [Cl'] = y + z.$$

According to the reasoning of the Donnan theory,

$$x^2 = y(y + z)$$

and

$$e = 2y + z - 2x$$

where e is the amount by which the total concentration of diffusible ions of the jelly exceeds that of the outer solution. The value e is thus a measure of the tendency for the ions of the jelly to diffuse into the outer solution to make concentrations equal in both the jelly and solution phases. But this could be accomplished only by the jelly swelling without limit, absorbing all of the solution. This, however, does not always happen. The chloride ion from the casein chloride can diffuse only by dragging its bulky casein cation with it. But the casein ions are bound by cohesive forces into an elastic jelly and these forces resist the pull of the chloride ions outward. So long as the elastic limit of the jelly is not exceeded, the effect of this pull will be merely to cause an increase in volume of the jelly directly proportional to e, which is a measure of the outward pull.

The pair of equations given just above may be solved simultaneously, thus

$$e = -2x + \sqrt{4x^2 + z^2}.$$

At the isoelectric point, the values of x and z are practically zero, making e and consequently the degree of swelling practically zero. Upon the addition of HCl, x and z assume measurable values, giving e a measurable value and causing the jelly to swell correspondingly. Now, if the value of x is increased without limit by adding increasing amounts of HCl, since z has a limiting maximum value determined by the satura-

tion point of casein for HCl, the value of e must decrease without limit after passing through a point of maximum, for

$$\underset{x=\infty}{\text{Limit}} \left[-2x + \sqrt{4x^2 + z^2}\right] = -2x + 2x = 0.$$

Loeb and Loeb[10] have shown that this is exactly what happens. Swelling occurs according to the Donnan equilibrium until the elastic limit of the jelly is exceeded, when the casein becomes completely dispersed. Apparently, swelling always precedes the solution of casein in acid solution.

In each of a series of experiments, Loeb and Loeb took a small number of granules of isoelectric casein, all of which would pass through a

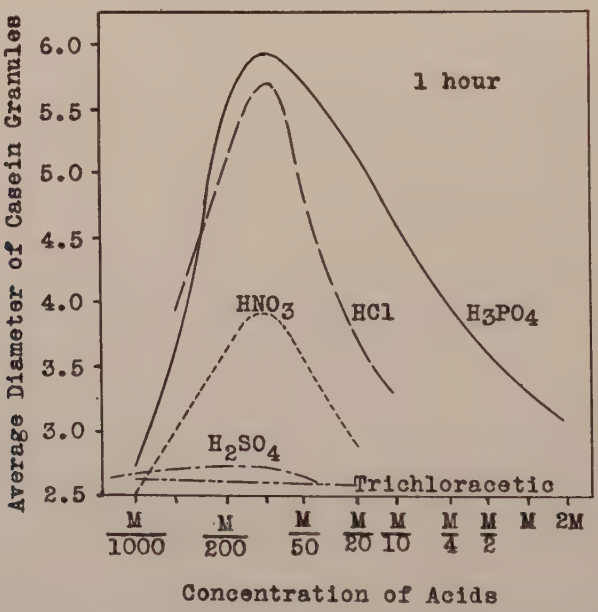

Fig. 2.—Swelling of casein in different acids at 24° C. in 1 hour.

100-mesh screen, but not through one of 120-mesh, and put them into 50 cc. of water containing various kinds and concentrations of acid and kept at 24° C. At intervals they measured the average diameter of about 15 granules in each test with a micrometer under a microscope. The relative diameter of the granules at the end of 1 hour is shown in Figure 2 as a function of kind and concentration of acid. Figure 3 shows the progress of the swelling at the end of 24 hours.

With increasing concentration of acid, the granules swell to an increasing extent, reaching a maximum at about pH = 2, and then the swelling decreases indefinitely, as predicted by the Donnan theory. Only in the region of greatest swelling does the casein pass into solution, indicating that the elastic limit of the casein has been exceeded and that the cohesive forces of the casein are no longer able to prevent what is practically swelling without limit.

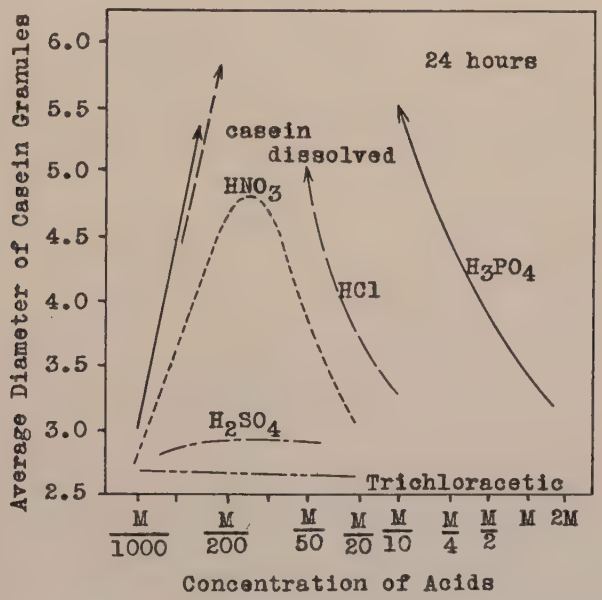

FIG. 3.—Swelling and solution of casein in different acids at 24° C. in 24 hours.

The graphs show that the rate of swelling is not the same for different acids. It was found that the rate of solution followed closely the rate of swelling. It took longer for casein to dissolve in nitric acid than in hydrochloric and casein appeared to be practically insoluble in sulfuric and trichloracetic acids when kept in them for 24 hours at 20° C.

The combination of casein with sulfuric and trichloracetic acids results in an increase in cohesion between the oily groups of the casein molecules so great that it cannot be overcome by the osmotic pressure due to the Donnan equilibrium and, consequently, in these acids casein swells but little and does not dissolve or disperse.

The dependence of the dissolving of casein upon swelling was also shown in the following experiment. One gram of isoelectric powdered casein, in each test, was put into 100 cc. of solution of HCl of different

concentration and kept for one hour in the first series and for 22 hours in the second. The mass was then poured into graduated cylinders and the undissolved part was allowed to settle to the bottom for two and for six hours, respectively, at 20° C. The supernatant liquid was removed and the sediment dried over night in an oven at about 100° C. The results are shown in Table I. The dry weight of 1 gram of isoelectric

TABLE I

AMOUNT OF CASEIN DISSOLVED AT 20° C. IN HCl SOLUTIONS OF DIFFERENT pH VALUES

pH Value	Milligrams Dissolved After One Hour	Milligrams Dissolved After Twenty-two Hours
4.36	42	102
3.32	55	133
3.11	86	164
2.97	249	267
2.94	265	342
2.84	—	459
2.75	—	536
2.64	348	634
2.53	408	646
2.36	—	733
2.18	547	788
2.06	538	779
1.87	401	710
1.66	366	528
1.50	272	374
1.40	219	300

casein was found to be 0.870 gram and this minus the dry weight of the sediment was the amount dissolved. The figures show that the rate of solution increases with diminishing pH value from 4.36 to 2.18 where the rate of solution of casein chloride is a maximum; with a further decline in pH value, the solubility diminishes again, like the swelling, and in agreement with the Donnan effect.

Effect of Salt

The Donnan theory enables us to predict the effect of adding a salt to the system which does not react chemically with the casein. Take the mono-monovalent salt MN and let the concentration of M^+ in the aqueous phase at equilibrium be represented by u. Then

$$e = -2(x+u) + \sqrt{4(x+u)^2 + z^2}$$

Since MN does not combine with the casein, its addition does not increase the value of z, but only of $(x+u)$, and it can be seen by inspection of the equation that this causes a decrease in the value of e. But e is the measure of the degree of swelling of the casein. Thus the addition of a

salt, like NaCl, to casein granules in hydrochloric acid must decrease the swelling in proportion to its concentration and thus retard or prevent solution. The data of Loeb and Loeb, plotted in Figure 4, show this

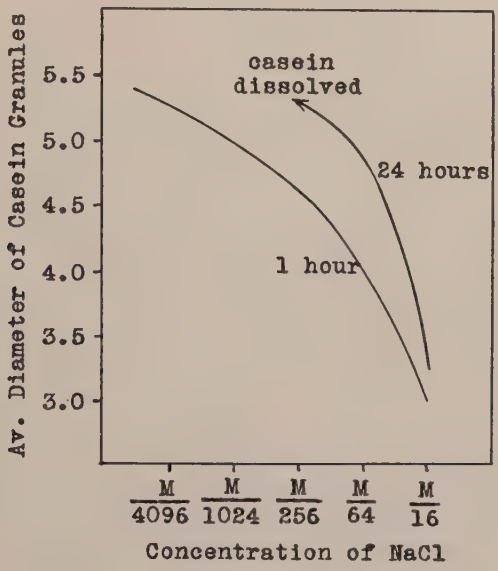

FIG. 4.—Depressing effect of salt on the swelling and solution of casein in M/100 HCl at 24° C.

actually to be the case. Table II shows the effect of NaCl on the rate of solution of casein in 0.0125-normal HCl. With increasing concentration of salt, there is a decreasing rate of solution corresponding to the decreasing swelling.

TABLE II

EFFECT OF CONCENTRATION OF NaCl UPON THE SOLUTION OF CASEIN IN 0.0125-NORMAL HCl AT 20°. (1 GRAM OF ISOELECTRIC POWDERED CASEIN IN 100 CC. HCl SOLUTION OF pH = 2.12)

	Concentration of NaCl					
Milligrams casein dissolved	M/2048 714	M/1024 685	M/512 665	M/256 615	M/128 449	M/64 282

Osmotic Pressure

If an aqueous solution of casein salt, say casein chloride, is separated from a protein-free solution by a membrane permeable to diffusible ions but not to protein ions, water will pass through the membrane from the

protein-free solution to the casein solution until the level of the latter is higher than that of the protein-free solution. This difference in levels at equilibrium is a measure of the difference in osmotic pressure between the two solutions. Such a system is a typical example of the Donnan equilibrium described above.

Loeb [11] demonstrated that the osmotic pressure shown by systems of casein chloride can be accounted for quantitatively by means of the Donnan theory. Portions of 1 gram of powdered casein were put into 100 cc. of water containing various quantities of 0.1-normal HCl. After 24 hours all the casein was dissolved in those solutions which contained more than 5 and less than 40 cc. of 0.1-normal HCl in 100 cc. The casein solutions were put into collodion bags, the opening of each of which was closed by a rubber stopper which was perforated by a glass tube serving as a manometer. Each bag was dipped into a beaker containing 350 cc. of water containing HCl to give it the same pH value initially as that of the casein chloride solution in the bag. Of course the concentration of acid did not remain the same on both sides of the collodion membrane. It is quite obvious from the discussion of the Donnan equilibrium that acid will at once start diffusing outward making the concentration of free HCl greater in the outside solution than in the casein chloride solution at equilibrium. The temperature was kept constant at 24° C. Osmotic equilibrium was usually established within 6 hours, but the final reading

TABLE III

DIFFERENCE IN pH VALUE AND OSMOTIC PRESSURE BETWEEN CASEIN CHLORIDE SOLUTION AND OUTSIDE SOLUTION AT EQUILIBRIUM

cc. 0.1-*N* HCl in 100 cc. of Solution	Appearance of Solution	pH Value		Osmotic Pressure mm.
		Casein Solution	Outside Solution	
1	precipitate	4.04	3.84	14
2	"	3.87	3.69	23
3	"	3.68	3.42	43
4	"	3.61	3.28	77
5	slight ppt.	3.46	3.13	135
6	solution	3.32	2.97	173
7	"	3.22	2.88	189
8	"	2.93	2.67	187
10	"	2.78	2.57	177
12.5	"	2.62	2.42	158
15	"	2.52	2.35	145
17.5	"	2.39	2.26	126
20	"	2.22	2.14	102
25	"	2.04	2.00	85
30	"	1.89	1.88	69
40	precipitate	1.73	1.73	41

was taken after 18 to 24 hours. Osmotic pressure was measured in terms of the height in millimeters of the solution in the tubes.

Table III gives the observed osmotic pressures and the pH values of both the casein solution and outside solution at equilibrium. The osmotic

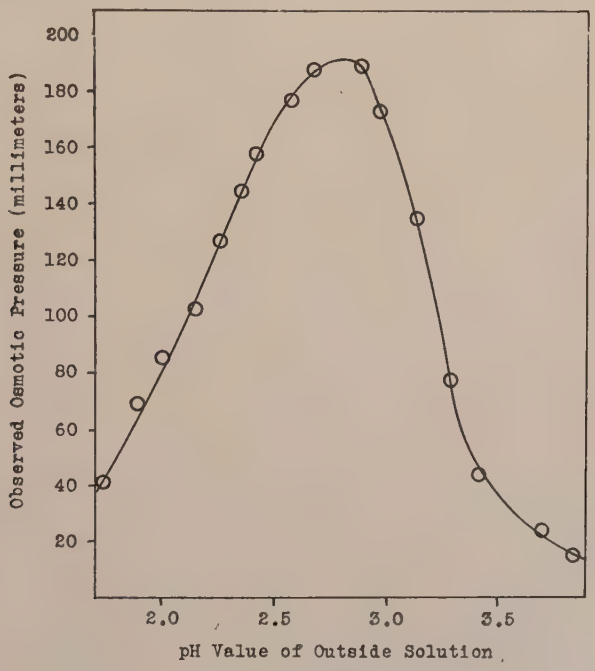

FIG. 5.—Osmotic pressure of casein chloride solution as a function of pH value of outside solution.

pressure is plotted as a function of pH value of the outside solution in Figure 5. The following calculations show how clearly the Donnan equilibrium accounts for the observed results.

In the outside solution, let

$$x = [H^+] = [Cl']$$

and in the casein solution in the bag, let

$$y = [H^+]$$

and

$$y + z = [Cl'].$$

The osmotic pressure is measured by the excess of concentration of diffusible ions in the casein solution over that of the outside solution and this is the value we have called e.

From the Donnan theory,

$$x^2 = y(y+z) \quad \text{and} \quad e = 2y + z - 2x.$$

Hence

$$e = (x-y)^2/y.$$

Since e is given in mols per liter, osmotic pressure in terms of e may be expressed as follows: $p = 250{,}000e$, where p represents the osmotic pressure in millimeters of water. Then

$$(x-y)^2/y = p/250{,}000,$$

from which we get

$$x = y + \sqrt{py}/500.$$

Thus, given y and p, we can calculate x. But Table III contains measured values for x as well as for y and p, enabling us to test the assumption that the Donnan equilibrium is responsible for the observed osmotic pressure by comparing the pH value of the outside solution calculated from observed values of y and p with actual measurements of pH value of the outside solution.* This comparison is given in Table IV and the

TABLE IV

Comparison of Observed Values for pH Value in Outside Solution with Those Calculated on the Assumption that the Osmotic Pressure Observed Was Due Entirely to the Donnan Equilibrium

pH Value of Outside Solution	
Calculated	Observed
3.79	3.84
3.61	3.69
3.40	3.42
3.28	3.28
3.11	3.13
2.98	2.97
2.84	2.88
2.67	2.67
2.56	2.57
2.44	2.42
2.36	2.35
2.26	2.26
2.12	2.14
1.96	2.00
1.83	1.88
1.69	1.73

closeness of the agreement between calculated and observed values leaves no doubt that practically all of the observed effect is due to the Donnan equilibrium.

The effect of adding salts to the system is similar to that described above for other systems. For example, according to the Donnan theory,

*Method suggested by D. I. Hitchcock.

the addition of salt would lower the value of e and, consequently, the observed osmotic pressure. This is exactly what happens. The addition of electrolytes that raise or lower the degree of swelling of casein jelly exerts a parallel effect upon the osmotic pressure of the systems just described.

Membrane Potentials

Another prediction made by the Donnan theory is that an electrical difference of potential across the membrane must exist in systems like those just described because of the unequal distribution of ions between the protein solution in the collodion bag and the outside solution, measured by

$$E = \frac{RT}{nF} \log_e \frac{x}{y} \text{ volts.}$$

In connection with the work on osmotic pressure just described, Loeb tested the Donnan theory in its application to membrane potentials in casein chloride systems. He made up 4, 3, 2, 1, 0.5, and 0.25 per cent solutions of casein having a pH value of 2.5 by adding HCl. These were put into collodion bags and each bag was immersed in 350 cc. of HCl solution having an initial pH value of 2.3. After 18 hours the pH values of the casein chloride solutions in the bags and of the protein-free outside solutions were measured by means of hydrogen electrodes.

In the equation given just above, $n = 1$ since HCl is a monobasic acid. Changing from natural to common logarithms and substituting the numerical value for RT/F at 24° C., we get

$$E = 59. \log (x/y) = 59 (\log x - \log y) \text{ millivolts.}$$

But $-\log x =$ pH value of outside solution and $-\log y =$ pH value of casein chloride solution. Hence the difference between the pH value of the casein solution and that of the outside solution multiplied by 59 should give the potential difference across the membrane in millivolts.

By a very ingenious arrangement, Loeb succeeded in measuring the membrane potentials predicted in the Donnan theory. His apparatus consisted essentially of a pair of saturated calomel electrodes, each having a capillary arm filled with a saturated solution of KCl. The end of one capillary arm was dipped into the casein solution in the collodion bag and the end of the other into the outside solution. The calomel cells were then connected to a Compton electrometer and the potential difference was measured. It was that of the cell:

calomel electrode	saturated KCl	external solution	membrane	casein solution	saturated KCl	calomel electrode

Everything else being symmetrical, the potential difference measured was that between the external solution and the casein solution, across the collodion membrane.

TABLE V

AGREEMENT BETWEEN HYDROGEN ELECTRODE POTENTIALS AND MEMBRANE POTENTIALS DETERMINED BY LOEB'S APPARATUS

Casein chloride, per cent	4.0	3.0	2.0	1.0	0.5	0.25
pH casein solution (A)	2.595	2.595	2.580	2.53	2.46	2.46
pH outside solution (B)	2.230	2.270	2.305	2.34	2.36	2.39
(A) minus (B)	0.365	0.325	0.275	0.19	0.10	0.07
H-electrode P.D., millivolts	21.5	19.2	16.2	11.2	5.9	4.1
Membrane P.D., millivolts	20.0	18.0	15.0	10.8	7.2	3.1

Table V gives the comparison between the potential difference as measured by the hydrogen electrode and as measured by Loeb's apparatus. The agreement strikingly illustrates that it is really a Donnan equilibrium that is being measured.

In a similar manner, an electrical difference of potential exists between the solution absorbed by a particle of casein jelly and the surrounding solution, and the same formulas apply. In dealing with the electrical properties of dispersions of casein, it is important to recognize this fact. E represents the measurable potential difference and not the absolute value of the electrical charge. In fact, if the absolute value of the charge, measured by z, remains constant while x is increased, the value of E will fall. At the isoelectric point the value of E is a minimum; with increasing value of x, E will increase to a maximum and then decrease indefinitely. Dispersions of casein will be most stable when E is at its maximum value and least stable when it is at a minimum value, as at the isoelectric point. Adding salt to a casein chloride solution lowers the value of E, thus explaining the mechanism of salting out.

Viscosity

The viscosity of casein solutions increases with the total volume of casein particles per unit volume of water. With increasing concentration of casein from 0 to 100 grams per liter, the viscosity increases from that of pure water to a value about 25 times as great. But casein particles are capable of increasing their volume by absorbing water and swelling. Thus if the particles of casein in a dispersion swell to twice their volume, the ratio of their volume to that of the free water is more than doubled and a corresponding increase in viscosity results.

The dependence of the viscosity of casein dispersions upon the swelling of the particles was demonstrated by Loeb [12] with a suspension of powdered casein in water containing different amounts of HCl. Figure 6

shows the ratio of the viscosity of the casein dispersion to the viscosity of pure water as a function of pH value after 1 hour and after 22 hours at 20° C. The 1-hour curve closely parallels that for the swelling of casein particles in HCl solution shown in Figure 2. With increasing swelling of the particles, there is a corresponding increase in viscosity. Figure 3 shows that the most highly swollen particles pass into solution

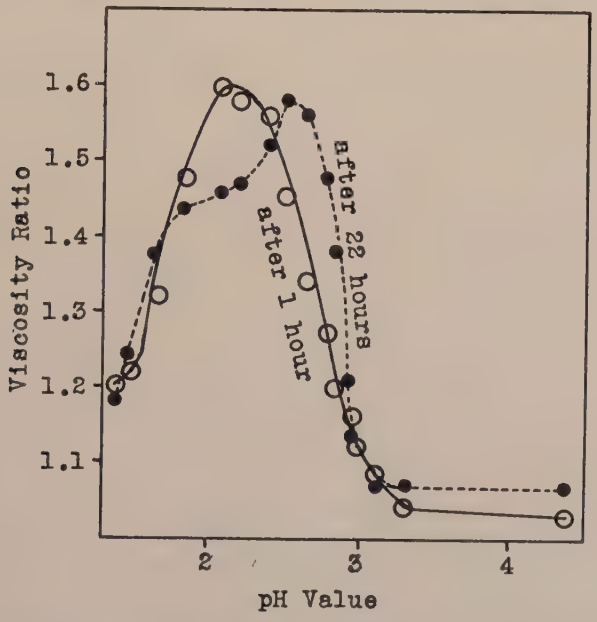

FIG. 6.—Viscosity of 1 per cent casein chloride solution after 1 hour and 22 hours at 20° C.

in the course of a day. This, of course, has the effect of releasing the water of imbibition and lowering the ratio of volume of casein to volume of free water, which in turn lowers the viscosity. This accounts for the drop in viscosity after 22 hours in the vicinity of pH = 2.2, the region of maximum swelling.

Ionic Mobility

When a direct current is passed through a solution of alkali caseinate, there is a deposition of casein at the anode. Greenberg and Schmidt [13] found that the amount of casein deposited is directly proportional to the current; *i.e.*, it obeys Faraday's law; and that the amount of casein deposited is inversely proportional (within the limits studied) to the amount of alkali which is combined with the casein. They obtained a

value of 2,015 for the equivalent weight of casein, in good agreement with Cohn's value, given above, considering the great differences in the methods used.

Greenberg and Schmidt used the Hittorf method to measure the transport numbers of the casein anion in solutions containing 5 to 6 cc. of added tenth-normal alkali per gram of casein and from these calculated the mobility of the casein anion. The alkalies used were NaOH, KOH, CsOH, and RbOH. The results are shown in Table VI. The mobility,

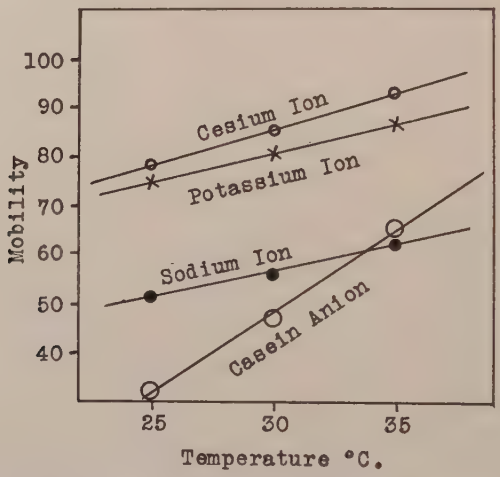

FIG. 7.—Mobility (or equivalent conductivity at infinite dilution) of the ions of alkali caseinate solutions as a function of temperature.

or equivalent conductivity at infinite dilution, is given for a temperature of 30° C. Values are also given for racemic casein.

The mobility of the ions of alkali caseinate solutions is pictured in Figure 7 as a function of temperature. The data of Greenberg and Schmidt can best be interpreted by the view that casein anions and inorganic cations are present in the solutions and that they act as the carriers of the electric current.

General

The foregoing shows how the mystery surrounding the chemistry of the proteins is being dispelled by the recognition that the reactions of the proteins really do follow the classical laws of the physical chemistry of amphoteric electrolytes, although complicated by the practically non-diffusible nature of the complex protein ions involved and their tendency to introduce more phases into the system by forming aggregates or setting to jellies.

TABLE VI

TRANSFERENCE NUMBERS AND MOBILITY OF THE CASEIN ANION

Cation Used	Transport Number Casein Anion	Mobility at 30° C.		
		Cation	Casein Anion	Casein Compound
Casein				
Sodium	0.453	56.2	46.2	102.4
Potassium	0.363	81.4	46.5	127.9
Cesium	0.334	85.3	43.0	128.3
Rubidium	0.355	84.5	45.5	130.0
Racemic Casein				
Sodium	0.390	56.2	37.0	93.0
Potassium	0.318	81.4	37.7	119.1

For a better understanding of the properties of casein, the entire literature of the physical chemistry of the proteins should be consulted. For a bibliography of the older work, Robertson's book [14] should be consulted. For more extended references to the application of the Donnan equilibrium to proteins, a study should be made of Loeb's book [9] and of Chapter V of the writer's book.[15] Reference should also be made to a paper by Hitchcock [16] on some consequences of the theory of membrane equilibria.

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Chapter 3

The Manufacture of Casein

By A. O. Dahlberg

Commercial skim-milk casein, to which the discussion in this chapter refers unless otherwise mentioned, is a creamery by-product, usually manufactured only when surplus skim-milk cannot be marketed in the form of foodstuffs, or profitably used for animal feeding. A large portion of the domestic casein is made during the few summer months of flush milk supply, when there is a surplus above the requirements for food manufacture. The seasonal production and the price obtained for casein have not encouraged improved and standardized methods of operation, each creamery usually adopting that one of the long used methods, or of the numerous modifications thereof, which is most easily adapted to its own conditions. The standardization of the methods now in use would have the very desirable result of yielding a more uniform product. Moreover there are very good possibilities for improving the general quality of casein made in this country through improved manufacturing methods.

Casein making is sometimes considered out of the question in a plant where other dairy products are made because of the objectionable odors believed to be a necessary result of the process. While too often casein is made under such conditions, they are by no means necessary. With proper equipment and the exercise of reasonable care commercial casein can be made in a plant with other dairy products without danger of impairing their quality.

The domestic production and the imports of casein for the last nine years are given in Table I.

The quality of casein depends upon the material from which it is made, the method of precipitation, and minor conditions of manufacturing operations. Most of the good quality casein is made from separator skim-milk which is as low as possible in fat, free from foam at the time of precipitating the curd, and manufactured without the use of higher temperatures than are necessary. Foam on skim-milk, when the curd is precipitated, gives trouble with insoluble particles in the dried casein and it should therefore be avoided.

TABLE I

DOMESTIC PRODUCTION AND IMPORTS OF CASEIN *

Year	Production	Imports
	Pounds	Pounds
1916	8,421,973	10,376,641
1917	8,342,350	12,319,111
1918	8,675,504	12,106,855
1919	13,924,836	8,125,175
1920	11,526,000	21,238,822
1921	8,076,000	9,717,238
1922	6,927,000	14,342,498
1923	14,548,000	26,489,992
1924	20,759,000	17,749,985

* U. S. Department of Agriculture figures.

Equipment Required

Much the same type of equipment has been used for a long time in making casein. With some of the manufacturing methods it would be advisable to improve the equipment, thus making possible a better quality of casein at a lower cost.

A complete outfit for making casein from skim-milk includes the following:

1. Precipitating vats.
2. Curd presses with divider boards and press cloths.
3. Suitable curd grinder.
4. Screen covered drying trays with trucks.
5. Drying tunnels with low pressure fan blower and heating coils.

With some of the methods drain racks and drain cloths are also required. In localities where the wet cooked curd may be shipped to one of the central drying plants maintained by the casein dealers, the only equipment required is the precipitating vat. Casein dealers are generally in a position to furnish the complete outfit necessary and to give reliable information concerning its installation.

Precipitating Vats.

Wooden vats are used almost exclusively because of their cheapness and the fact that they are not attacked by acids. Vats, holding from four to six thousand pounds of skim-milk, are made up in rectangular shape, similar to a cheese vat, and are provided with gate valves at one end for draining off the whey. With some of the methods, a larger vat, fitted with a mechanical agitator, can be used to advantage as it will reduce the labor cost and allow much faster handling of the skim-milk, besides

giving more favorable conditions for the control of quality. For making rennet and grain curd casein the Golden State Milk Products Company of California design and build large, round-bottom, wooden precipitating vats with mechanical agitators. These are capable of handling 19,000 pounds of skim-milk at one time, and have proven very satisfactory from the standpoint of saving time and labor and giving better control and more uniform quality.

Curd Press.

The curd press must be well constructed to withstand the heavy strain to which it is subjected, and in addition it should be so constructed as to make it easy to keep it clean.

A very satisfactory press, simple in design and easily kept clean, which is used extensively on the Pacific Coast, is made as follows: Two surfaced planks, 1¾ inches by 13 inches by 6 feet, made of clear, strong lumber, are placed on edge, parallel to each other. At right angles to them, flush with the ends, are fastened two cross pieces 2¾ inches by 9½ inches by 4 feet, allowing each end of the cross pieces to project 2 inches. On the opposite side of the 6-foot planks, two more cross pieces are attached in the corresponding positions. The projecting ends of the cross pieces are then bolted together with ½ by 15-inch machine bolts, using heavy washers. Two ½-inch holes should be bored in the projecting ends of each cross piece, 1¾ inches from the end and 1½ inches from each edge. This will bring the bolts close to the upright pieces. The frame is then bolted lengthwise with four ¾-inch machine bolts, 6 feet 4 inches long, bolt holes having been bored through the center of the 9-inch dimension of the cross pieces, 7 inches from each end. This will leave approximately 33 inches between the upright bolts. Large cast iron washers, or better still, iron plates ½ by 4 by 18½ inches with two 1-inch holes centered 1⅜ inches from each end, are used on top and bottom of the bolts, and the nuts on top are drawn up sufficiently to take the strain which will be exerted by the press screw. A press screw, 1¾ by 30 inches, is fastened to the under side of the top cross pieces with a heavy iron plate or 3½-inch piece of hardwood to throw the pressure against them when the press is in use. Lag screws are used to fasten the plate to the bottom of the cross pieces. A press built according to this description is shown in Figure 1.

A 2¾ by 16½ by 35-inch piece of strong material, with a 1-inch square cut out of each corner for the upright bolts, is used for a follower board and guide just under the swivel cap of the press screw. The follower board, which is taken out when the press is not in use, helps greatly in getting the curd to press evenly and maintains the press screw in proper alignment. If a shelf, 8 inches wide by 32 inches long, with

slightly tapered ends, is added to each side of the bottom cross pieces, flush with their tops, it is of much help in holding the curd in place when it is being put in the press and before pressure is applied.

Two pieces, 2 by 4 by 18½ inches, inserted beneath and at right angles to the bottom cross pieces, next to the long bolts, raise the press from the floor and act as skids for moving the press.

Courtesy of R. A. Simpson Mfg. Co.

FIG. 1.—Curd Press.—Form divider and press boards shown in press.

The press boards required at the bottom and on top of the curd cakes are made 30 inches square, of double 1½-inch surfaced material nailed cross grain to each other. The cross boards distribute the pressure evenly and protect the light press racks between the curd cakes.

The lumber used in making the press and press boards should be well oiled with raw linseed oil either before or after assembling, preferably before. Oiling prolongs the life of the equipment and keeps the press boards from becoming heavy through the absorption of moisture.

A press of the dimensions given will handle the curd from five to six thousand pounds of skim-milk at one time.

Press Form-Dividing Boards.

The dividing boards, which are placed between the layers of wet curd in the press, can be readily made from ¾ by 1½-inch strips surfaced on

all sides. The strips are cut in 30-inch lengths and nailed at right angles to one another, as shown in Figure 2, allowing approximately 1 inch between strips, so that the dividing board is 30 inches square and suitable for the press described. Divider boards made of hardwood strips ½ by 1⅜ by 30 inches are very serviceable and take up less space. A smaller space than 1 inch between the strips makes washing difficult, while a much larger one allows the curd to project, which causes unsatisfactory pressing. It is best to use good clear wood which can be washed easily, and to give the boards at least two coats of raw linseed oil before using,

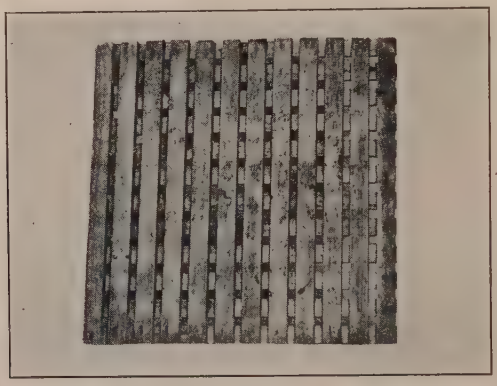


FIG. 2.—Divider board used between curd cakes in press.

to retard the absorption of water by the wood. The boards are kept wet while in use and unless well oiled will soon become heavy with absorbed moisture. They should be washed each day with hot water and a brush to prevent offensive odors. Washing may be done either in a wash sink or on the floor, using a floor brush and hot water from a hose.

Drain and Press Cloths.

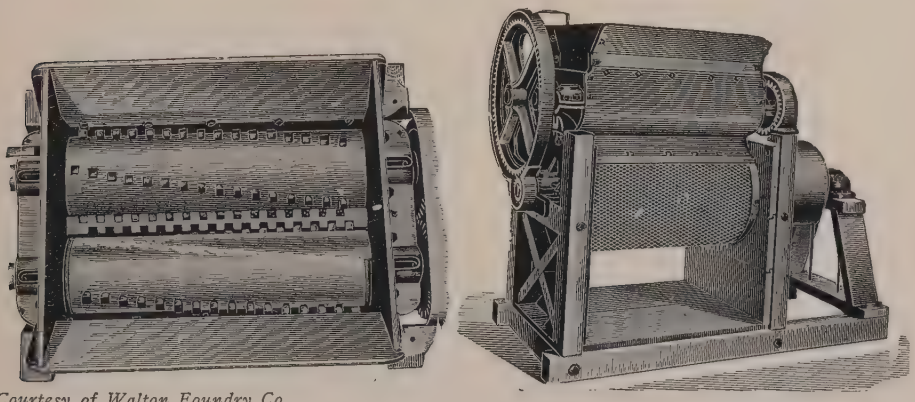
The drain cloth must be strong and closely woven so as to retain the heavy, wet curd without tearing in manipulating and yet allow of easy drainage, and be easy to wash. Burlap makes a strong, serviceable drain cloth but it is harder to wash and may not allow as good drainage as some of the other materials. A coarse weave marquisette or scrim is excellent as it gives good service and is easier to wash than burlap. The drain cloth should be made large enough to extend over the sides and ends of the drain rack, and the cut edges should be hemmed to prevent raveling.

For pressing the wet curd in the press 10-ounce duck is very satisfactory because it has great strength, is easy to wash, and will not allow

any of the curd to escape. A closely woven burlap may be used, but it is much harder to wash. Two cloths are generally used for each cake of curd, which eliminates sewing and makes washing easier. The cut ends of the cloths should be hemmed.

Curd Mill.

It is important to get the right kind of mill for grinding the curd, for if it is not ground properly the most favorable drying conditions cannot be obtained. In order to do satisfactory work the mill should exert both a shredding and a beating action. In the better machines, the shredding action is obtained by means of a comparatively slowly-revolving



Courtesy of Walton Foundry Co.

FIG. 3.—Curd mill for cooked curd.

cylinder fitted with sharp-peg teeth which tear the large masses of curd into small pieces. These fall into a lower cylindrical drum, in which a rapidly revolving iron beater drives the curd through a medium-mesh screen. Especially strong mills are required for cooked curds because they are very tough and hard to grind. A mill which is very satisfactory for this type of curd is shown in Figure 3.

Drying Trays and Trucks.

Satisfactory drying trays can be made of ½ by ⅞-inch wooden strips and galvanized screening. A rectangular frame is made of the strips which are mortised and glued at the corners. Heavy galvanized screening, 14 to 16 meshes to the inch, is tacked to one side of the frame, after which ½-inch by ⅞-inch strips are placed over the screen, flush with the lengthwise strips and fastened to them with brass screws. Another

½ by ⅞-inch strip 30 inches long is fastened to the bottom, parallel to and half way between the side strips, by means of a screw at each end. The strips forming the bottoms of the trays strengthen them materially but do not interfere with free circulation of air when they are stacked on the trucks. The construction of the drying trays is shown in Figure 4.

The wear and tear on the wooden drying trays, due to handling and to the drying of the wood in the heated air, together with the trouble from splinters, nails and screws getting into the product, suggests the use of more satisfactory and durable trays. It is reported that all metal trays

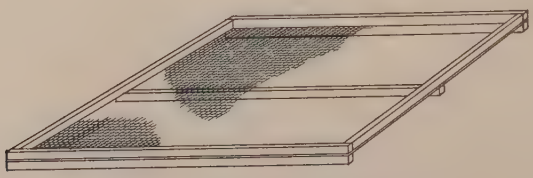


FIG. 4.—Drying tray for casein.

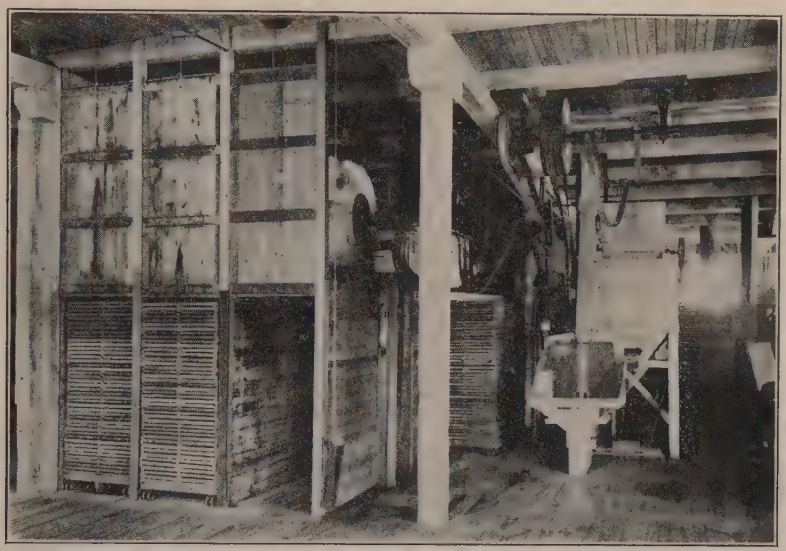
have been tried out under commercial conditions with very satisfactory results.

The trucks for holding the drying trays are made the same size as the trays of 2 by 4-inch surfaced lumber, or other suitable material, with mortised joints and are provided with a swivel caster at each corner. The number of trucks and trays needed depends to a large extent upon the time in which the casein is to be dried. With a well pressed and ground curd, properly spread, a 30 by 30-inch tray will hold the equivalent of 1½ to 2 pounds of dried casein. Twelve wooden trucks and 30 dozen 30 by 30-inch trays should be available to handle 20,000 pounds of skim-milk daily. Due to quantity production the trays and trucks can be purchased already made up at a price which may be lower than they could be made up for locally.

Tunnel Drier.

The best type of tunnel drier is made of tongued-and-grooved boards nailed to both sides of studding, the space between being filled preferably with dry sawdust or other insulating material. It is advisable to line the tunnels with galvanized iron if they are to be used a great deal, as the large volume of heated air passing through them dries the boards out to a considerable extent. A cheaper type of construction consists in nailing tongued-and-grooved boards on only one side of the studding, which, although not so economical because of leaks and losses, gives satisfactory results, as far as the drying of the casein is concerned. It is more economical of both space and construction to have two or more tunnel driers

built together with one wall in common, and located where they will be least in the way. The tunnel should be made 1 inch wider than the trucks and drying trays to allow sufficient room for moving the stacked trays freely through it. Most tunnels are made 31 or 32 inches wide to accommodate the stock size 30-inch-square drying trays which can be purchased ready-made. A double-tunnel drier, 22 feet long, 6 feet high, and each tunnel 31 inches wide, when filled with drying trays will hold the casein from 30,000 pounds of skim-milk. The driers can be made of any



Courtesy of Golden State Milk Products Co.

FIG. 5.—Tunnel drier showing trays on trucks. Fan blower and heating coils on top of drier.

length and height but it is advisable to have them high enough to allow a man to walk in without inconvenience when pushing the trays in or out. Full-width, vertical, sliding doors are placed on each end of the tunnels so that the trucks stacked full of trays may be pushed in at one end and out of the other, which facilitates and systematizes the work.

Blower and Heating Apparatus.

A low pressure fan type blower is used for forcing the heated air through the tunnels. It should be of sufficient size to provide the necessary volume of heated air to pass over the material on the trays, and connected with heating coils of ample radiating surface to heat the air to the temperature required. With a drying temperature of 130° to

135° F. it is recommended that air be supplied at the rate of four cubic feet per minute, per pound of wet curd per hour going into the drier.[1] For the best results it is advisable to have the blower and heating coils somewhat oversize rather than too small. Even under such conditions the volume of air can be easily regulated with dampers.

Reliable information on the size of blower and heating coils may be obtained from the manufacturers of this equipment by furnishing them with data on the conditions and the quantity of curd to be dried in a given time.

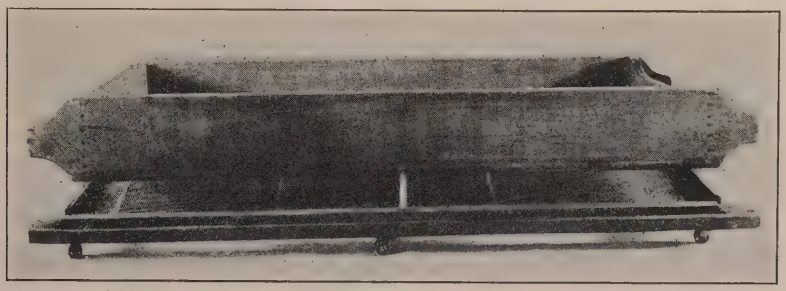
Drain Racks.

Drain racks such as are used in the manufacture of cottage cheese are very satisfactory. They should be made either of cypress or of a good grade of white pine as these woods are light and stand the moisture very well. A frame 3 feet 4 inches wide by 6 feet 4 inches long, outside dimensions, is made of 2-by-4-inch surfaced material, using mortised joints and allowing the ends of the longer pieces to project 3 inches. The frame is made with the 2-by-4-inch pieces lying flat. On the inside edge of each piece a ⅞-inch hole about 2 inches deep is bored every 12 inches, and a ½-inch galvanized pipe of the proper length placed in the holes when putting the frame together. The pipes support the wire screening and keep it from sagging and getting loose. Three galvanized swivel casters on each side of the bottom of the frame allow the drain rack to be handled conveniently. Extra heavy galvanized-wire screening of four meshes to the inch is nailed to the top of the frame with staples, taking care to draw it tight and let it extend a little over the middle of the frame all around. A wooden strip, wide enough to cover the edge of the galvanized screening, is nailed around the outside edge of the upper part of the frame to hold the top part in proper position. The top part is made of 1½-by-12-inch surfaced material with mortised and spiked corner joints, each end of the side pieces projecting 6 inches. The projections, trimmed down to the shape of a handle, make the rack more convenient. Figure 6 shows a completed drain rack.

Two drain racks of the dimensions given are large enough to hold the curd from about 5,000 pounds of skim-milk. The curd drains quickly, permitting the racks to be used repeatedly until all the casein is made. One of these racks may at times be used to advantage in any of the methods in which they are not ordinarily employed, for occasionally conditions are encountered which give considerable fine curd, a large amount of which may be lost in the whey if draining facilities of ample capacity are not available.

When making casein on a large scale requiring drain racks it is advisable to have them constructed to operate back and forth on a stationary

track which will allow of their being rolled under the vat to receive the wet curd. (See Figure 13.) The curd in the drain rack may then be rolled back for further washing, and when drained, quickly unloaded by having the drain rack pivoted in the wheeled frame at a center point at



Courtesy of U. S. Department of Agriculture.

FIG. 6.—Drain rack on casters for convenience in moving.

each end in such a manner as to allow of its being inverted. Such an arrangement necessitates elevating the vats somewhat, but the many advantages more than warrant it where a large quantity of casein is made.

Methods of Manufacturing

While there are various methods of making commercial casein they are much the same in their general procedure and differ mainly in the manner of precipitating the curd and in the amount of care exercised in the work. Local conditions largely determine the method to be used. If the whey is to be utilized for the recovery of milk sugar hydrochloric (muriatic) acid must be used, as the sulfates formed when sulfuric acid is used seriously interfere with the manufacture of milk sugar. The whey from curd produced by allowing the milk to sour itself is not satisfactory for making milk sugar.

When the whey is not saved for the recovery of milk sugar, and when sufficient vat space is available, some modification of the self sour method is quite generally used, because of the possible saving in acid and the general preference of the trade for this type of casein.

Casein to be used in the preparation of food products should be made only by special methods and under strict sanitary conditions.

Essentially the manufacture of casein consists of precipitating, washing, pressing, grinding, and drying the curd of skim-milk,—the operations being carried out in the order mentioned. The methods of precipitating the curd differ greatly from the standpoint of the type of curd secured, some being much more favorable to washing out the excess acid and

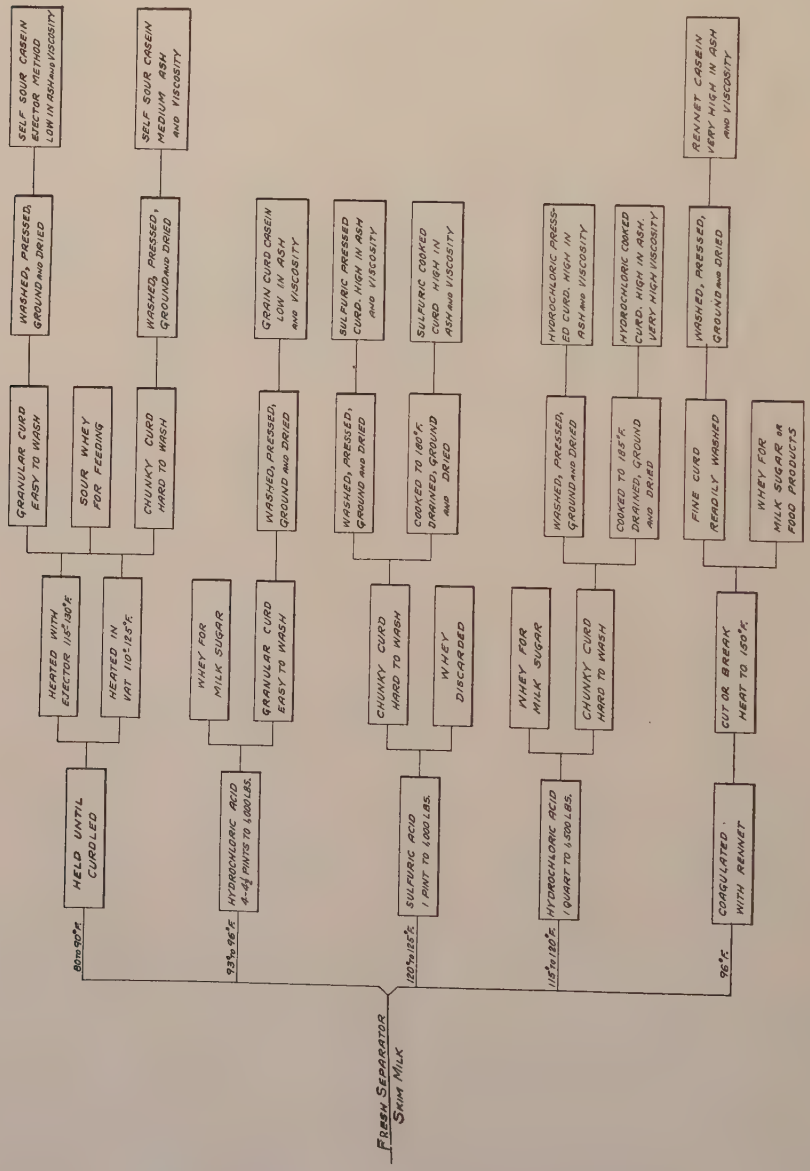

FRESH SEPARATOR SKIM MILK
80 to 90°F.
93° to 98°F.
120° to 125°F.
115° to 120°F.
96°F.
HELD UNTIL CURDLED
HEATED WITH EJECTOR 115° 130°F.
HEATED IN VAT 110° 125°F.
HYDROCHLORIC ACID 4-4½ PINTS TO 1,000 LBS.
WHEY FOR MILK SUGAR
GRANULAR CURD EASY TO WASH
SULFURIC ACID 1 PINT TO 1,000 LBS.
CHUNKY CURD HARD TO WASH
WHEY DISCARDED
HYDROCHLORIC ACID 1 QUART TO 1,500 LBS.
WHEY FOR MILK SUGAR
CHUNKY CURD HARD TO WASH
CUT OR BREAK HEAT TO 130°F.
COAGULATED WITH RENNET
GRANULAR CURD EASY TO WASH
SOUR WHEY FOR FEEDING
CHUNKY CURD HARD TO WASH
WASHED, PRESSED, GROUND AND DRIED
WASHED, PRESSED, GROUND AND DRIED
WASHED, PRESSED, GROUND AND DRIED
COOKED TO 180°F. DRAINED, GROUND AND DRIED
WASHED, PRESSED, GROUND AND DRIED
COOKED TO 185°F. DRAINED, GROUND AND DRIED
FINE CURD READILY WASHED
WHEY FOR MILK SUGAR OR FOOD PRODUCTS
SELF SOUR CASEIN EJECTOR METHOD LOW IN ASH AND VISCOSITY
SELF SOUR CASEIN MEDIUM ASH AND VISCOSITY
GRAIN CURD CASEIN LOW IN ASH AND VISCOSITY
SULFURIC PRESSED CURD, HIGH IN ASH AND VISCOSITY
SULFURIC COOKED CURD HIGH IN ASH AND VISCOSITY
HYDROCHLORIC PRESSED CURD, HIGH IN ASH AND VISCOSITY
HYDROCHLORIC COOKED CURD, HIGH IN ASH, VERY HIGH VISCOSITY
RENNET CASEIN VERY HIGH IN ASH AND VISCOSITY
WASHED, PRESSED, GROUND AND DRIED

FIG. 7.—Sequence of operations in casein making.

impurities than others. The ordinary sulfuric and hydrochloric (muriatic) acid methods in general use give a curd collecting in large lumps, difficult to wash, and resulting in a less desirable grade for some uses.

Figure 7 shows in diagrammatic form the principles and sequence of operations in making casein by the various commercial methods.

During the world war the Grain-Curd method [2] for making a better and more uniform grade of pressed curd, acid casein was developed by the Federal Bureau of Dairying to meet the requirements of the Aircraft Production Department of the Army. This method, while somewhat more expensive, proved so satisfactory from the standpoint of giving a better and more uniform grade of casein that a modification of it is still used by one of the largest manufacturers of casein in this country.

The casein made by the methods generally used may be greatly improved in general quality and uniformity by the exercise of reasonable care and attention in the manufacturing process. This is too often not given because casein is considered a cheap by-product from which little profit may be realized.

Since the procedure following the precipitation and washing of the curd is essentially the same for so many of the methods, it will be convenient to discuss it first in a general way, leaving to later sections the description of the several precipitation processes and the variations in the details of the subsequent operations which they may entail.

Draining and Washing.

After the precipitation of the curd, the whey is drained off through the gate valve of the vat. With the methods in which no drain rack is used, the whey as well as the wash water should be drawn off through a drain bag, tied to the gate valve, to prevent unnecessary loss of curd particles. The curd is pushed back from the gate valve before opening the latter so as to leave as much as possible of it in the vat.

Washing of the curd is important and should not be neglected with any of the methods. In washing the curd in the vat with water the mixture must be stirred well to remove as much of the acid and whey as is consistent with the type of curd. When precipitating at the higher temperatures with sulfuric or hydrochloric (muriatic) acid large chunks of curd are secured which are not favorable for efficient washing. Even under such conditions, washing, if carried out as recommended, will materially improve the product.

The washed curd is drained well, then pressed, ground, dried and packed for shipment.

Pressing.

A bottomless form 24 inches square and 8 inches deep made of 1-inch surfaced boards is placed at the bottom of the press rack and lined with

two press cloths of proper size. Extending both ends of opposite boards about three inches and fastening a three-cornered piece of material in the corner so formed will strengthen the form and make it more durable. After placing the proper quantity of wet curd in the form the cloths are neatly turned over from both sides so as to hold the curd securely when pressure is applied, special care being given to the corners, where trouble is most likely to occur. The form is then removed and a dividing board placed on top of the mass of wet curd. The form is then placed on the top of the dividing board and the process continued until all the curd has been taken care of. The amount of curd that may be

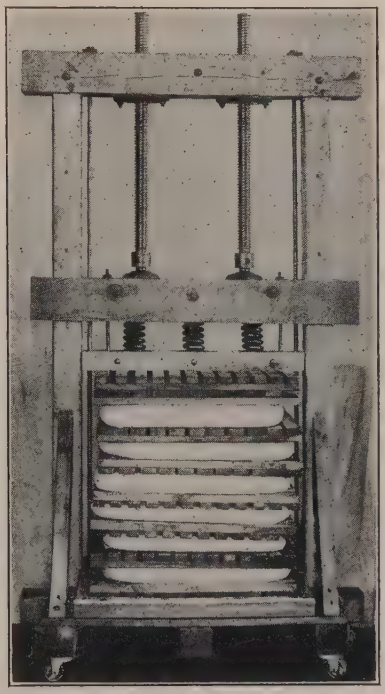
Courtesy of Golden State Milk Products Co.

FIG. 8.—Battery of curd presses in a California plant.

satisfactorily pressed in one cake will depend upon its condition. A quantity giving a well pressed cake 2½ to 4 inches thick will ordinarily be most satisfactory. Pressed curd may contain from 50 to 65 per cent moisture, depending upon the type of curd and the degree of pressing. A battery of curd presses in a commercial plant is shown in Figure 8.

When all the curd has been placed in the cloths pressure is applied by means of the press-screw until it is sufficiently dry for satisfactory grinding. This will require repeated tightening of the press screw, as the expulsion of moisture from the curd under pressure quickly releases the tension. A satisfactory device for maintaining continuous heavy pressure, where someone is not at the plant all the time to tighten the presses, can be readily made by means of heavy railway-car springs, as shown in Figure 9, and added to the regular curd press. When pressure is first applied to the curd sufficient attention should be given to see that

the cakes are pressing evenly; afterwards the press-screws have to be tightened only occasionally. If heavy pressure is applied too quickly,



Courtesy of U. S. Department of Agriculture.

FIG. 9.—Curd press showing application of car springs.

the curd cakes may start pressing unevenly, and require removing and re-arranging to secure satisfactory pressing.

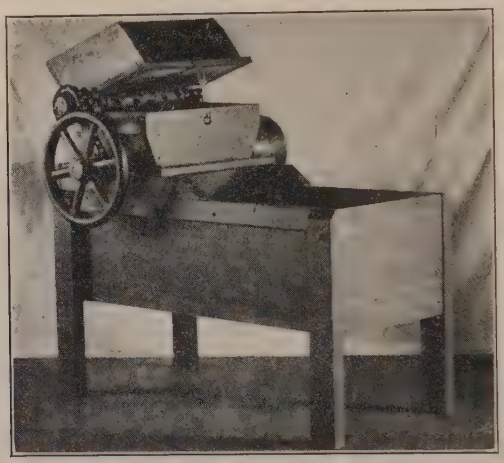
Grinding.

The curd should not be taken from the press until it is sufficiently dry for proper grinding and drying. When pressing has been ample no trouble will be experienced in grinding the curd to a fine, dry, mealy state, in which it spreads well and does not cohere in lumps which prevent rapid and thorough drying. When insufficient moisture is pressed from the curd serious difficulties are experienced, as it is extremely hard, if not impossible, to grind a wet, soggy curd. Ground curd, if wet, cannot be spread satisfactorily, and coheres in lumps which dry on the surface to a hard, impervious, bone-like state, while the interior remains moist.

Properly pressed and well ground curd has the following advantages:

1. It requires less steam and dries more rapidly.
2. It gives casein of better appearance and brighter color.
3. The capacity of the drier is increased.

The curd mill shown in Figure 10 is inexpensive and has proved satisfactory for handling all except cooked curd.



Courtesy of U. S. Department of Agriculture.

FIG. 10.—Curd mill for other than cooked curd.

Spreading Ground Curd on the Drying Trays.

The finely ground curd is most easily spread upon the drying trays shortly after grinding. While the work is usually done by throwing a shovelful of the ground curd on a tray and then spreading it out with the hands, better results can be obtained by the use of a simple shaker screen, similar to those used for screening sand and gravel. A box arrangement that is supplied with ground curd by means of a hopper permits the empty trays to be pushed under the screen. Such a box can be quickly made at very little cost, and will save time and also spread the curd in a uniform layer which affords the best drying conditions. When the curd is not spread uniformly the drying time is increased to the time necessary to dry the thickest parts. The casein should not be spread too thickly or the time of drying will be unnecessarily long, which gives a poorer color and may otherwise affect the quality. As the trays are filled they are stacked upon wooden trucks provided with swivel casters for convenience in running them in and out of the drier.

Drying.

The stacked trays with the ground curd uniformly spread upon them are run into the tunnel drier where a current of heated air is passed over them. The time required for proper drying depends on:

The condition of the curd, with particular regard to its moisture content and fineness of grinding.

The uniformity of the spreading on the trays.

The volume and temperature of the air passing over the curd.

The volume and speed of the air current blowing over the curd should not be excessive, but sufficient to carry the moisture off readily. When the air current is too swift, much of the fine dried casein is blown off the trays.

The use of a return flue on the tunnel drier for recirculating a portion of the heated air through the drier is economical from the standpoint of heat units required for removing the moisture. While the heated air passing over the wet curd will be pretty well saturated with moisture at the outset, as the drying progresses it could be recirculated through the tunnel drier to effect a material saving. Table II, showing the temperature and humidity of the air in a tunnel drier during the process of drying casein, indicates the possibilities in this direction. The writer has seen one such casein drier in successful operation where the air was recirculated over the burning coke used for heating.

A temperature of 115° to 130° F. (46.11° to 54.44° C.) depending on the type of curd, is sufficiently high for all purposes. Higher dry-

TABLE II

TEMPERATURE AND HUMIDITY OF AIR DURING PROCESS OF DRYING CASEIN IN TUNNEL DRIER *

Interval	Condition of Air			
	Tunnel Inlet		Tunnel Outlet	
	Temp.	Humidity	Temp.	Humidity
	° F.	Per cent	° F.	Per cent
Start of run	127	0.5	64	66
After 1 hour's drying	120	9.0	100	33
" 2 " "	124	8.0	105	26
" 4 " "	128	11.0	114	17
" 6 " "	120	15.0	120	15
" 7 " "	124	12.0	124	12

* Unpublished data from tests made by J. T. Bowen, Bureau of Dairying, U. S. Dept. of Agriculture, on commercial tunnel drier. Average air velocity in tunnel 1,558 f.p.m. Average volume of air passed through tunnel, 2,454 cubic feet per minute.

ing temperatures are very likely to cause objectionable discoloration of the casein, especially in combination with some of the other influencing factors. The reddish brown color, commonly regarded as burnt casein, is due to drying poorly washed curd at too high a temperature. A higher temperature may not be objectionable with a well washed curd, but the difficulty of washing sufficiently the large chunks of curd from the usual sulfuric and hydrochloric acid methods makes it inadvisable to use a temperature above the range mentioned. Casein dried at the proper temperature and time comes from the drier in a fine-grained condition and breaks up very easily, while that dried at a higher temperature has more tendency to cohere in one mass and is harder to grind.

Drying should be completed without any interruptions, during which the moist curd may start molding and otherwise deteriorating. The partially dried curd molds very readily in warm weather and this may cause a great deal of trouble. Moldy curd gives rise to very disagreeable and offensive odors, is difficult to dry, sticks to the trays to contaminate the next lot of curd spread on them, and gives a lower quality, bad smelling casein which is not altogether soluble.

Microscopic examination of the insoluble white flakes sometimes found in casein solutions shows that they often consist of masses of mold hyphæ, and it has proved possible to obtain these in enormous quantities by allowing moist casein to mold intentionally. Their origin in casein, therefore, seems fairly well established.

In order to get a product that will keep well it is important to dry the casein thoroughly before it is removed from the drier. It is an easy matter to ascertain when a properly ground curd is dry, but great care must be exercised in determining when a coarsely ground one is ready to be taken from the drier. Since large particles of curd dry slowly they must be broken or cut in two to find out whether the inside is properly dried. Provided the curd has been well ground and spread upon the trays in the proper manner, no trouble need be experienced in drying the casein completely within 6 to 8 hours at a temperature of 115° to 130° F. (46.11° to 54.44° C.).

The dried casein is collected by inverting and jarring the trays over a bin or a hopper connected to an elevating device. The empty trays are restacked on another truck. The casein comes from the trays easily if the pressing and grinding have been well done, otherwise it has a tendency to stick to the trays somewhat.

While the tunnel drier has been the only type used to any extent in making commercial casein in this country, considerable work has been done on the development of a more satisfactory drier for the purpose. One of the most recent ones, which appears to give promise of satisfactory results in commercial operation, is a drier invented by Mr. E.

Foster of Australia.[3] In this apparatus the pressed curd, in chunks weighing about a half pound, is forced through a vertical conduit pipe by means of a current of heated air. The curd is broken by its impact against an obstruction plate; automatically returned to the starting point by gravity, and the operation of forcing it through the conduit pipe and against the obstruction plate continued until drying is completed. The obstruction plate, preferably concave, and with its concave side down, is fastened to the roof of a circular container within another chamber. The wall of the inner container has a 40-mesh screen around its waist, through which the curd passes to the outer chamber, when sufficiently reduced in size and properly dried, and to the graders for bagging.

The fine dust particles of casein produced during drying are recovered by filtering the exhaust air from the outer chamber through a silk bag or other suitable device.

The inventor claims that the more even and thorough application of heat to the surfaces of the particles prevents fermentation of the wet curd and discoloration of the finished product.

Grinding and Packing Casein.

Grinding dried casein gives it a more uniform grain and appearance and a lighter color, and allows a greater weight of it to be packed in the same size bag. Some dealers, however, prefer to buy the unground casein, as it gives them a better idea of the manufacturing conditions and permits them to grind it to suit their requirements.

There are several types of mill which are used satisfactorily for grinding casein. An ordinary burr feed grinder can be used, but is not as satisfactory for heavy duty as some of the other types.

In grinding casein a magnet of sufficient strength to collect nails, screws, or other pieces of iron, should be used in the feed pipe to prevent their getting into the grinder and injuring it. Such pieces of metal may also come through the grinder and ruin the bolting cloth when a bolter is used.

If the ground casein is to be sold on the basis of a certain mesh it should be bolted, and the coarse particles returned to the grinder. The finished casein is packed in grain sacks or lined burlap bags for shipment.

Yield of Casein.

The yield of casein will depend upon the composition of the skim-milk and the method and manner of manufacturing. It may vary between 2.75 and 3.5 per cent, but with skim-milk of average composition the yield of well dried casein usually ranges between 3 and 3.25 per cent. The condition of the separated curd and the care of handling may have a great deal of influence on the yield. There is necessarily oppor-

tunity for greater losses, and reduced yield, with the methods giving a finer curd, and requiring more handling during separation, washing and draining, as in the case of the self-sour, ejector and grain-curd methods. When properly handled any of the methods will give a normal yield for the composition of the skim-milk used. In changing over several plants making large quantities of casein from the older hydrochloric (muriatic) acid pressed curd, precipitated at a higher temperature, to the grain-curd method the writer found no difference in the average yields obtained, when the work was properly done.

Some fine curd may result in the acid pressed and acid cooked curd methods from the use of too much acid or an improper method of adding it, as well as from incorrect temperature regulation. The yield of cooked curd casein, especially, may be materially reduced by the addition of too much acid. This causes much fine material which is easily lost with the equipment ordinarily used in making this type of product.

Self-Soured or Lactic Acid Methods

The self-soured or lactic acid type of casein is usually regarded by the trade as the best quality for general use because of its better solubility, greater strength, and lower viscosity when dissolved. The precipitation of the curd is secured by the development of lactic acid in the skim-milk through the normal fermentation of the lactose by bacillus lactic acidi, and the application of heat to the curdled skim-milk. Various modifications of the method are in use, depending upon local conditions.

Vat Method.

The fresh skim-milk from the separator is allowed to stand in wooden vats at a temperature favoring the development of lactic acid, and when the process is to be completed as soon as possible, from five to ten per cent of sour whey is added to hasten fermentation. A portion of the whey from which a previous lot of curd has been separated is held over from day to day at a temperature of 90° to 110° F. (32.2° to 43.3° C.) to provide a supply of the lactic acid bacillus for inoculation.

When the skim-milk has developed sufficient acidity to give a soft curd, as shown by physical examination, it is heated by means of direct steam while stirring vigorously with a wooden rake until a clear separation of the curd and whey is secured. A temperature from 110° to 125° F. should give the desired results, with the curd collecting at the bottom of the vat in a more or less coherent mass, leaving the whey clear and free from small particles of curd. The clear whey is drained off through a drain bag tied on the gate valve to catch curd particles which might otherwise be lost, and the required amount is saved and

soured for the next day's use. The curd is washed by covering with cold water and stirring well. After draining away the wash water it is ready for pressing.

Too great acidity of the skim-milk at the time of heating will give a finer curd, harder to handle without the use of a drain rack, while insufficient acidity will prevent a clear and efficient separation of the curd. Experience will soon teach the operator the proper acidity and conditions for obtaining the best results. With a good active whey starter, used in the quantity mentioned, warm skim-milk should develop within a few hours' time sufficient acidity for securing a clear separation of the curd when heated.

When the skim-milk is allowed to stand over night, as is quite often done, the temperature at which it is held and the quantity of whey starter used should be so regulated as to prevent the coagulated skim-milk from standing too long before heating. The heating should be done shortly after the skim-milk is coagulated in order to secure a curd which can be handled most easily. If the curdled skim-milk is allowed to stand for some time, a higher heating temperature will have to be used to get the curd in satisfactory condition for handling without a drain rack, but it should not exceed 135° to 140° F. (57.22° to 60.0° C.) in any case. Stirring more carefully when heating highly acid skim-milk will lessen the breaking up of the curd into fine particles.

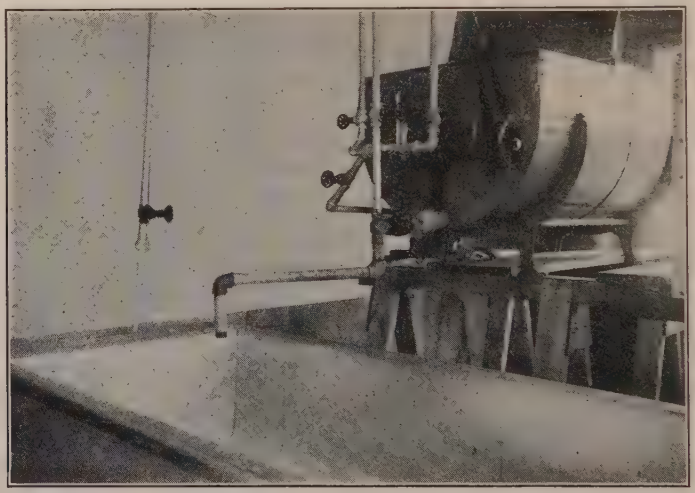
Ejector Method Self-Sour or Lactic Casein.

Heating well-soured skim-milk with a firm curd by passing it through a steam ejector in transferring it from one vat to another will give a rapid separation of granular curd very favorable for washing.

The fresh skim-milk is pumped into an elevated vat and allowed to sour until a firm curd has developed. Sour whey may be used to facilitate the development of acidity, or the skim-milk allowed to sour of its own accord. If heating with the ejector is attempted before the skim-milk shows a firm coagulation, a tough, rubbery curd, impossible to handle properly, will be secured. When sufficient time is not available for securing a good firm curdling of the skim-milk, the vat self-sour method should preferably be used. Undue agitation of the curdled skim-milk, such as might result from pumping, gives a much finer curd when heated with the ejector. This is harder to handle and some of it may be lost through the drain cloth.

The elevated vats in which the skim-milk is soured should be sufficiently high to allow the curdled skim-milk to run by gravity to the ejector and thence into another vat below, under which the drain rack, mounted on casters, may be placed. This arrangement permits the use of just what steam is required for separation, makes handling more convenient, and

requires the least labor. If the skim-milk is lifted by the ejector the steam required may be so great as to result in too high a temperature. The size and number of ejectors to use will depend upon the quantity of sour skim-milk to be handled. Since all the heat necessary for separation is applied while the skim-milk is passing through the ejectors time will be saved by having them of such size that the desired temperature may be reached quickly. A valve placed in the skim-milk pipe leading from the tank to the ejector, and one in the steam pipe next to the ejector, permit easy control of the temperature to which the skim-milk



Courtesy of U. S. Department of Agriculture.

FIG. 11.—Arrangement of ejector and vats in making ejector-method casein.

must be heated to obtain proper separation. Figure 11 shows the arrangement described for the ejector and vats.

The temperature necessary to obtain a clear and rapid separation of the curd from a lot of skim-milk depends upon the acidity and condition of the curd; it can be ascertained by taking samples of the heated skim-milk as it comes from the ejector. By varying the temperature used, the proper degree of heat necessary to get the best results may be easily ascertained. At that temperature the curd rises to the top quickly, leaving the clear whey below.

Because of the rapid separation in the lower vat, the whey can be draining away through the gate valve and lined drain rack while heating the curdled skim-milk as it passes through the ejector on its way from the upper to the lower vat. This permits a saving in time and allows a smaller lower vat to be used. When the whey has drained

away the curd is washed in the vat by covering with water which makes it firmer and facilitates handling. The curd is then placed in the drain racks where it may be washed further before pressing. The use of a nozzle on the end of a water hose is advantageous in washing the curd on the drain rack, for with it the massed curd can be broken up better. Draining is hastened by occasional manipulation of the drain cloth. Two good washings of the more or less granular type of curd secured by this method will give a very good grade of casein. After the last washing the curd is allowed to drain well before pressing. The curd handles very well and is not as tough and hard to grind as that made with the usual sulfuric and hydrochloric (muriatic) acid pressed curd methods.

Sour Whey Method.

This method of precipitating the curd by means of lactic acid depends upon the development of high acidity in whey held over from the preceding day in quantities sufficient to obtain a continuous separation of the curd when it is mixed with the skim-milk as fast as it is received from the separator. The sour whey and the skim-milk, each heated to a temperature of 130° to 135° F., are drawn off into a common pipe or trough where they mix in proper proportions to produce curdling, and then discharge into the bottom of a small box or other receptacle placed in a drain rack lined with a drain cloth. The use of a steam jet or ejector for heating the skim-milk, and valves in the whey pipe from the elevated tank and in the skim-milk supply pipe will allow of securing the proper combination of skim-milk, sour whey and heat to give the required separation of the curd. The overflow from the box is taken care of by the drain rack, which allows the clear whey to drain away and retains the curd. Sufficient whey for the following day's run is pumped into an elevated vat for further souring. The accumulated curd in the drain rack is either pressed in the usual manner or allowed to drain until the following morning, when it can be ground for drying without the necessity of pressing, providing sufficient heat and sour whey were used at the time of precipitating.

Sulfuric Acid Casein

Pressed Curd Method.

The fresh skim-milk is heated in the vat with direct steam to a temperature of 120° F. (48.89° C.) and never higher than 125° F. (51.67° C.). A solution of sulfuric acid, made by adding 1 pint of the commercial acid (Sp. gr. 1.83) to two quarts of water, is then added to each 1000 pounds of fresh skim-milk. Stirring must be continuous while the acid is being added, until a good separation of the curd is obtained,

as indicated by the clearness of the whey. This requires but a few minutes providing sufficient acid has been used. In case the curd does not separate well, and leave a clear whey, more acid is gradually added to get the proper results, but no more than is required to give a good separation of the curd should be used. This will vary according to the strength of the acid and the condition of the skim-milk. The curd is pushed back from the gate valve and after draining off the whey is covered with cold water and washed by stirring well. After draining off the wash water the curd is ready for pressing or it may be handled by the cooked curd method.

Cooked Curd Method.

This method eliminates the pressing of the curd and requires practically no equipment other than the precipitating vats. It may be adopted when the creamery wishes to sell wet curd and is located where it can be shipped to a central drying plant. Cooked curd casein gives a solution of much higher viscosity than self-sour casein when dissolved with certain solvents and for this reason is not favored for some uses.

The process of precipitating is the same as that used for pressed curd; that is, the skim-milk is heated to 120° F. (48.89° C.) and sulfuric acid (diluted in water) added at the rate required to cause proper separation. After draining off the whey the curd is broken up in the vat, covered with water, and the mixture heated to a temperature of 170° to 175° F. (76.67° to 79.44° C.) by means of direct steam. At that temperature all the curd should collect in a semifluid, plastic, tough mass. The water is drained off and after kneading the soft curd to remove as much moisture as possible, it is placed in a barrel where it settles into an almost air-tight mass, which, upon cooling, becomes very tough and impervious and in that state will keep for several days, even in hot weather. Fresh-cooked curd can be put on top of the hardened curd in a barrel partially filled from a previous batch. Barrels filled with cooked curd and covered with burlap can be shipped to a central drying plant without danger of breaking. Cooked curd is very hard to grind and requires an especially strong mill.

No more acid than is required to give a clear separation should be used, as an excess seriously interferes with the proper working of the cooked-curd method. When too much acid is added the curd, when cooked in the water, does not collect in the characteristic plastic mass but breaks up into fine particles, making it difficult to handle. When the proper quantity of acid has been used in precipitating the curd, the clear whey shows a titrable acidity of from 0.30 to 0.32 per cent using phenolphthalein as indicator. If it shows more than 0.34 per cent acidity, trouble is very likely to be encountered in obtaining the characteristic

fusion of the curd at the time of heating. A good plan to follow in avoiding the use of an excess of acid is to measure out what the directions call for—one pint per 1,000 pounds of skim-milk—dilute all of it with water and then add a portion at a time, using only what is necessary to effect a clear and complete separation.

When the cooked curd is to be dried at the creamery where it is made, time and expense may be saved by handling it in some other manner than packing in barrels. Where sufficient clean cement floor space is available the hot plastic curd may be deposited in batches and allowed to spread out on the floor in flat chunks that can be handled sooner and more easily. The curd may also be allowed to remain in the bottom of the vat to drain until ready for grinding if the vat is not needed for additional batches. The use of sloping drain boards will facilitate the drainage of the whey from the cooked curd, which, after draining in the vat, is cut into chunks of suitable size with a shovel and placed on the drain boards to complete draining.

Hydrochloric (Muriatic) Acid Casein

Pressed Curd Method.

The fresh skim-milk is heated to a temperature of 115° to 120° F. (46.11° to 48.89° C.), never exceeding 120° F. (48.89° C.), as the higher temperature will give a much less desirable casein. Commercial hydrochloric (muriatic) acid of 20 to 23 Baumé is then gradually added while stirring continuously until precipitation of the curd is secured. Approximately 2½ pints of acid (diluted by pouring into an equal quantity of water) will be required for every 1,000 pounds of skim-milk. Care should be taken not to add acid beyond the point giving the best separation since an excess will give a soft curd, hard to handle. Adding the diluted acid gradually and stirring well after each addition will make it easier to avoid using too much.

The hydrochloric (muriatic) acid curd precipitated by this method is inclined to be soft and may give trouble by sticking to the press cloths. To overcome this difficulty the curd from which the whey has been drained as well as possible is covered with lukewarm water, and a few ounces of sulfuric acid (well diluted with water) for every 1,000 pounds of skim-milk, which will make the curd firmer and easier to press after draining off the water.

Acid Cooked Curd Method.

This method, which is similar to the sulfuric acid cooked curd method, likewise eliminates the necessity for pressing the curd. The process of precipitating the curd is the same as that for the pressed curd but the

treatment with the sulfuric acid water need not be carried out. The curd is covered with cold water and heated with direct steam to a temperature of 175° to 185° F. (79.44° to 85.0° C.) which will bring about the fusion of the curd into a semifluid, plastic, tough mass, providing too much acid has not been used. The same factors influencing the proper working of the method, mentioned under sulfuric acid cooked curd, apply equally to this method. After draining off the water the plastic curd is kneaded to remove as much moisture as possible and handled as described under sulfuric acid cooked curd casein.

Grain-Curd Casein

The grain-curd method was developed and put into commercial operation in several large factories by the United States Department of Agriculture, Bureau of Dairying, during the World War, for the purpose of obtaining a uniform and high quality casein to meet the demands for airplane construction. It is particularly well suited when the whey is to be used for the recovery of milk sugar. By precipitating at a lower temperature, and at a definite acidity, using much more dilute hydrochloric (muriatic) acid than was formerly employed, a granular curd is secured, which permits of thorough and quick washing with water acidified to the same degree as in the precipitation of the curd.

It was found when hydrochloric (muriatic) acid in about normal concentration was added to fresh skim-milk, at a temperature of 34° C. (93.2° F.), until the expressed whey indicated an apparent pH of 4.6 with methyl red, that the curd had a remarkable granular character,[2] and a resiliency which permitted of extensive washing without the packing which ordinarily occurs. While requiring additional equipment in the form of drain racks and drain cloths the method is highly desirable from the standpoint of better quality casein and the easier pressing and drying of the curd.

The granular condition of typical grain-curd makes it possible to handle it in a centrifuge. Zoller reports [4] from tests carried out under commercial conditions that typical grain-curd is particularly adapted for handling in a large factory centrifuge. With it, he claims, the operations from the precipitating vat to washing and pressing can be made entirely mechanical, making possible a saving in time, an improvement in sanitary conditions and the use of less equipment.

Indicator Solution and Color Standards.

Since it is necessary to work at exactly the right pH, accurate control must be maintained by the use of standard buffer solutions and methyl red indicator. The use of the color standards necessary for de-

termining the acidity for precipitating the curd and for acidifying the wash water is no more difficult than the making of an ordinary titration. Standardized color solutions and the proper indicator are easily obtained. The indicator solution may be purchased already made up or it may be prepared by dissolving 0.2 gram of pure methyl red in 1,000 cc. of 95 per cent grain or wood alcohol. This 0.02 per cent solution gives a deep color when five drops are added to 10 cc. of standard or unknown mixtures. Standard buffer solutions of pH 4.6, 4.8 and 5.0, for making the color standards required may be purchased from chemical supply houses.

The color standards are prepared by pouring 10 cc. of the standard buffer solution of the desired pH into a graduated test tube, and adding 0.5 cc. of the methyl red indicator solution. One such color standard is made with each of the three buffer solutions. Test tubes of uniform thickness and diameter, and graduated for 10 cc. may be purchased for this particular work. The color standards should be marked to show the pH represented and should be kept well corked and paraffined to guard against contamination. Methyl red is not at all stable, and as the color standards fade quickly they should be replaced frequently. It is extremely difficult to tell when fading has taken place to a serious extent, and for this reason a safe rule is to make up new standard weekly.

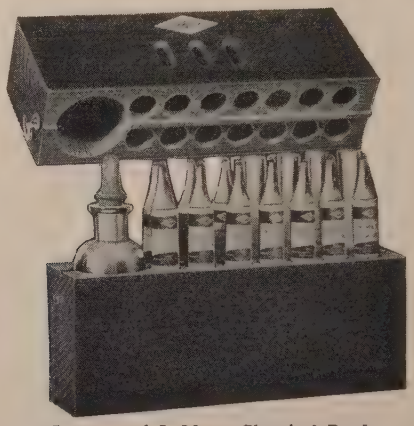
Instead of methyl red it may prove desirable to use bromcresol green, one of the more recent indicators, because of its stability in the form of color standards. A set of these standards, covering the pH required, may be purchased at a low cost and with proper care may be used for a year's time without replacement. The bromcresol green may show a slightly different pH from the methyl red but any such variations can be determined by a comparison of the two indicators in commercial practice and the proper pH for the bromcresol green ascertained in this manner. Work * done in comparing the apparent pH colorimetrically with methyl red and bromcresol green, and on the relation between these results and the actual pH as determined by the electrometric method, indicates good possibilities in the use of bromcresol green.

In using the color standards with a turbid solution like whey a comparator block or set should be used for greater accuracy. A convenient and inexpensive comparator set may be purchased or can be made by boring two rows of holes of proper size lengthwise in a block of wood of convenient size and then boring cross holes through the middle of three of them in a manner to allow of looking through the contents of three tubes. Such a comparator set is shown in Figure 12.

In making determinations by comparison with the color standards

* Work done through the courtesy of Miss Anne G. Benton, Bureau of Dairy Industry, Washington, D. C.

three test tubes are filled to the 10 cc. mark with the whey to be tested and placed in the back row of the observation holes. To the middle tube add 0.5 cc. of the methyl red indicator and place a tube of distilled water in front of it. Place the pH 5.0 and 4.8 color standards,



Courtesy of LaMotte Chemical Products Co.

FIG. 12.—Comparator set for pH indicators.

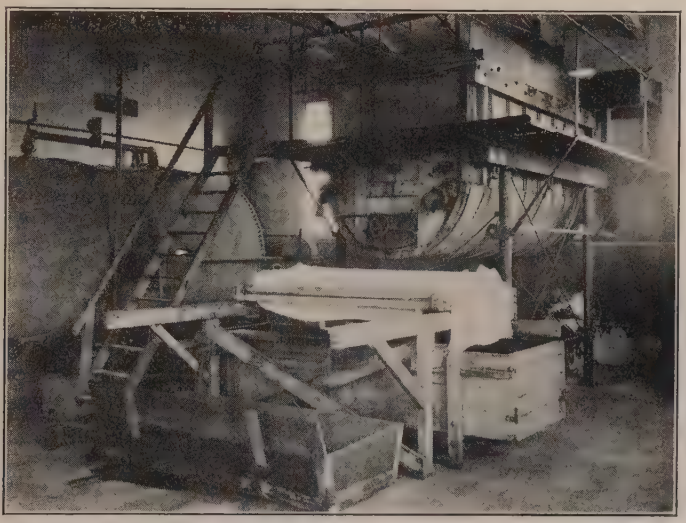
previously prepared, in the two remaining holes, and compare the colors of the three pairs of tubes. By this arrangement the effects of color and turbidity are eliminated and accurate results obtained.

Precipitating the Curd.

The temperature of precipitation of the curd is a very important factor in securing the typical, granular character which makes it easy to wash. A temperature of 94° F. (34.44° C.) gives the desired results with reasonably fresh skim-milk; if it is slightly sour a temperature of 93° F. should be used to prevent the clumping of the curd into larger chunks, which are difficult to wash. While the temperature will have to be varied slightly, it should under no circumstances be above 96° F., as above this limit typical grain-curd will not result.

Hydrochloric (muriatic) acid (diluted with eight times its weight of water) is added slowly, preferably by distributing it along the full length of the tank by means of a wooden trough with many small holes bored through the bottom, while stirring rapidly to incorporate it thoroughly and secure the desired separation. When the skim-milk starts to curdle the acid should be added more slowly but with no let up in stirring. When sufficient acid has been added to secure a good separation, leaving a clear whey, the curd is allowed to settle in the vat, and about

half of the whey drained off through the drain cloth in order to economize in the amount of acid needed in the next operation. The remaining volume of whey is then brought to a definite end point of acidity by adding more acid in small amounts to the curd and whey mixture in the vat, stirring thoroughly, but not vigorously, to break up any clumps of curd, until 10 cc. of the whey with 5 drops of methyl red when compared with the standard solutions shows an apparent pH of 4.8 to 4.6. This acidity gives a granular curd which is firm to the feel and does not stick together when a handful is squeezed.



Courtesy of Golden State Milk Products Co.

FIG. 13.—Large precipitating vat with mechanical agitator and drain rack on wheels.

Care must be taken not to add too much acid. Frequent testing of the whey and examination of the curd will be necessary until the operator has become thoroughly familiar with the method. If the comparison with the color standards shows the whey sample, when observed as explained under "Indicator Solution and Color Standards," to have a red color equal to that of the pH 4.6 color standard too much acid will no doubt have been added. Methyl red reaches its full red color at pH 4.6 and further addition of acid will not be indicated by the color reaction. An experienced man can tell pretty well by feeling of the curd when sufficient acid has been added, provided the temperature of the skim-milk was correct. Too much acid gives a sticky curd, while too little gives a soft curd, either of which makes washing and draining difficult. Too low a precipitating temperature gives a soft curd, while

too high a one gives a tough curd collecting in clumps, both of which are undesirable.

The grain-curd method with Pasteurized milk gives a softer curd, which is hard to drain. This can usually be overcome by precipitating at a higher temperature.[4] The Pasteurizing temperature and time of holding determine the precipitating temperature to use for securing a good grain-curd. The following are given by Zoller as approximate requirements:

Pasteurization	Requires
63° C. for 30 minutes	40° C.
63° C. for 60 minutes	45° C.
63° C. for 100 minutes	50° C.
80° C. for 60 minutes	50° C.
100° C. for 100 minutes	52° C.

Sulfuric acid diluted with water so as to give an acid of about the same strength as the diluted hydrochloric (muriatic) acid may be used for making grain-curd casein but requires a precipitating temperature of about two degrees higher to secure a granular curd of the proper consistency. If the temperature is not increased the curd will be too soft and hard to handle.

Washing the Curd.

The remainder of the whey is now drained off, retaining as much of the curd in the vat as possible. The curd is then covered with water which has been brought to an acidity of pH 5.0 to 4.8 with hydrochloric (muriatic) acid. The wash water is made up by adding hydrochloric acid to water in small amounts at a time, testing 10 cc. portions after each addition by adding 5 drops of methyl red indicator and comparing the color with that of the standard solutions. After the first tank of wash water has been made in this way, subsequent supplies can be made up very quickly because the proportion of acid to use will be known very closely. As methyl red indicator reaches its full red color at a pH of 4.6 care must be exercised to avoid adding too much acid. Acid should be added only until a faint pink color is shown. The use of too much acid in the wash water has the same ill effects as the use of too much acid in precipitating. The curd is given a thorough washing by stirring it gently but in a manner that will insure breaking up any matting that may have occurred when the curd particles settled to the bottom. In draining off the wash water the curd is run onto the drain rack and further washing completed there, that the vat may be used again sooner. Additional washing on the drain rack by means of a hose connected to the wash water tank may be done while precipitating the next vat of skim-milk.

Pressing and Drying.

The curd from the grain-curd method requires slightly more attention at first than ordinary curd, due to its giving up moisture so readily. Pressure should be lighter at the outset and then attended to more frequently to maintain a heavy pressure. While requiring additional attention proper pressing of grain curd can be done in much shorter time and more moisture can be pressed out.

The grinding and drying of the curd are carried out in the usual manner. Properly made grain curd grinds and dries exceptionally well, giving a product of remarkably good color and less hard and flinty than the usual muriatic and sulfuric acid pressed curd casein.

Rennet Casein

Rennet casein to possess the properties desired by the trade must be made from absolutely fresh, low fat, skim-milk, before it has had a chance to develop any appreciable acidity. This type of casein is used extensively in the manufacture of casein plastics for which it must be carefully made, in a manner to insure of its meeting the strict requirements of this rapidly developing industry. This will necessitate using good equipment and keeping it all scrupulously clean. The users of rennet casein usually require that the product meet certain standards, the most important features of which are that it shall have an ash content of not less than 7.5 per cent, fat of not more than 1.0 per cent, a good bright color, and be free from all particles of dirt or foreign material of any kind.

Practically all of the rennet casein used by the American manufacturers of casein plastics is imported from France, New Zealand, and Australia, very little being made in this country.

Precipitating the Curd.

The perfectly fresh skim-milk is set at a temperature of 96° F. (35.55° C.) with sufficient rennet, properly diluted with water, to bring about the required coagulation in 15 to 20 minutes. The quantity of rennet required will vary, depending upon its strength and the condition of the skim-milk. With perfectly fresh skim-milk the quantity will range from 70 to 100 cc. for each 1,000 pounds of skim-milk, while for skim-milk in which a slight development of acidity has taken place the quantity will range from 40 to 60 cc. The time of cutting or breaking the curd and heating is important and will have to be modified to meet conditions. A good rule to follow is to note the time, from the addition of rennet to the first signs of coagulation, and add approximately one-tenth, or whatever fraction experience shows to give the desired results, to this

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period, as the time for starting to cut or break up the coagulated curd. Some directions for making rennet casein recommend continual stirring of the skim-milk during coagulation, or heating to a higher temperature than that mentioned. Heating of the mixture, either by means of a steam jacketed vat, or with direct steam, properly admitted by means of an arrangement to prevent the curd burning onto the pipes, is commenced shortly after starting to cut or break up the curd, and continued until the temperature reaches 150° F. (65.55° C.). After allowing the curd to settle for ten minutes the clear whey is drained off as quickly as possible through gate valves on the end of the vat. A series of gate valves will be of help in draining off both the whey and the wash water from the top down while the curd is settling. The curd is immediately covered with water at a temperature of about 75° to 80° F. and the mixture stirred for several minutes, taking special care to loosen all curd from the bottom of the vat. After standing for several minutes the water is run off into the cloth lined drain rack, and the process of washing repeated once or twice, keeping the temperature of the curd between 80° and 90° F. (26.66° and 32.22° C.), as a temperature lower than this has a tendency to give a product which is harder when dried. The curd remaining in the vat is run onto the drain rack with the last wash water and allowed to drain.

Pressing and Drying.

Extra care must be exercised in the pressing and drying of rennet curd because it gives up moisture quite readily. It should be pressed heavily for an hour and then ground and spread thinly on the drying trays to facilitate quick drying at a temperature of 110° to 115° F. (43.33° to 46.22° C.). A higher temperature or slow drying will give a dark, undesirable color.

Close attention to details is required to make a good quality of rennet casein, but by handling the skim-milk when it is very fresh and taking the proper precautions, no real difficulty should be encountered in producing a very satisfactory product.

Buttermilk Casein

The high fat content and the great variation in the physical condition of most buttermilk offer difficulties in making a good quality casein and have limited the production of a grade that can be used in place of skim-milk casein. It is generally sold at a much lower price and used in animal and poultry feeds, where its high fat content is an advantage and its darker color of no consequence. The best and most economic way of handling buttermilk is to dry it and recover all the solids for poultry and animal feeding.

Making casein from a mixture of skim-milk and buttermilk may give a satisfactory product but may also lower the quality of the entire product because of the condition of the buttermilk. The quality of casein secured from a mixture of buttermilk and skim-milk will depend largely upon the quality of the buttermilk used. A satisfactory product cannot be made by using a large proportion of buttermilk from Pasteurized, high acid cream.

Sweet Cream Buttermilk Casein.

Buttermilk from sweet cream butter can be easily and readily made into a marketable grade of casein by the ejector method of precipitating the curd described under directions for making skim-milk casein. The ejector method already described was originally developed in connection with the making of casein from sweet cream buttermilk,[5] for which it is particularly well adapted. It has been used for making considerable quantities which were sold readily at a price almost as high as that received for a good grade of skim-milk casein. It gives a fine, granular curd which can be easily and thoroughly washed, resulting in low ash casein.

Because of its high fat content the sweet cream buttermilk preferably should be separated before it is made into casein. This can be done without any difficulty when the product is in the normal condition after making sweet cream butter. Removing as much of the fat as possible will improve the quality of the casein and should be economical from the standpoint of butterfat recovered. The sweet cream buttermilk is allowed to sour by standing undisturbed until it develops a smooth firm curd. Undue agitation of the curdled buttermilk before heating will give a finer curd, harder to handle and recover fully.

The temperature of heating for obtaining the characteristic rapid and clear separation of the curd is within a range of 115° to 125° F. (46.11° to 51.66° C.). A higher temperature than is necessary gives a softer curd, harder to drain and handle, particularly with a high acid buttermilk, which may even re-emulsify. Buttermilk which has between 0.65 per cent and 0.80 per cent titrable acidity gives a curd that will handle well in every respect.

The curd from which the whey has been drained should be well washed with water, which can be done best in the drain rack required for this method. Washing with cold water makes the curd firmer and easier to handle, in addition to improving the quality of the casein. Buttermilk casein should not be dried at a temperature higher than 130° F. 54.44° C.) as the combination of the high fat and other influencing factors may result in a much poorer grade.

Sour Cream Buttermilk.

In making casein from sour cream buttermilk the latter is allowed to develop considerable acidity. It is then heated to a temperature which experience has shown to give the most favorable conditions for handling, and after allowing it to stand over night, or until the curd has separated from the whey, the latter is drained off by means of a series of gate valves at different levels in the end of the vat and the soft curd run into a drain rack which is lined with a sufficiently finely woven drain cloth to hold the curd and allow the whey to drain through. The draining of the curd may be slow, hence a sufficient number of drain racks must be available.

The curd from Pasteurized sour cream is so extremely fine that it is very difficult to handle and recover without resorting to methods which are too expensive, considering the value of the product obtained.

Composition, Physical Properties and Quality of Casein as Effected by Manufacturing Methods

The practical value of casein depends upon its solubility, adhesiveness, and general working properties. It should be low in fat, dissolve within certain limits of alkalinity and temperature of heating, possess good adhesive strength, viscosity, and working properties when dissolved, and be free from sediment or scum.

There is a wide variation in the composition and physical properties of casein made by the different methods, due to the influencing factors of the manufacturing methods. The variation may or may not be objectionable, depending upon the use to which the casein is to be put. The composition and properties of casein determine its adaptability for different uses.

There is quite a definite relation between the composition, especially the ash, and the physical properties of casein. The ash content in general indicates the viscosity, or water-absorbing properties, and in conjunction with the "acidity" gives a pretty good idea as to method and conditions under which it was made. Work done at the Forest Products Laboratory [6] showed that the ratio of water to casein required to give a solution of a definite viscosity increases in proportion to the ash content.

The ash and "acidity" of casein, two factors having a pronounced effect upon its properties, are largely dependent upon the method and manner of manufacturing. In order to secure casein of the composition and properties desired manufacturing methods providing the necessary conditions should be used.

The self-sour or lactic ejector method provides conditions for making a casein law in ash, "acidity" and viscosity. The development of

TABLE III

ANALYSES OF CASEINS *

Type	No. Samples	Times Washing Curd	Moisture	Fat	Ash	Nitrogen	Acidity	
							Not Corrected	Corrected for Ash
Specially Prepared			Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Self-sour ejector method	5	0	7.55	0.30	2.46	14.65	9.02	2.58
Self-sour ejector method	7	3	8.53	0.34	1.78	15.12	8.06	0.93
Sulfuric acid pressed curd	10	0	8.01	0.29	4.27	14.20	8.24	3.68
Sulfuric acid pressed curd	10	2	7.33	0.17	4.73	14.52	7.58	3.48
Hydrochloric acid pressed curd	3	0	6.57	0.16	5.76	14.20	7.60	4.60
Hydrochloric acid pressed curd	3	2	7.64	0.16	5.73	14.45	5.83	2.80
Sulfuric acid cooked curd	10	–	8.89	0.12	4.25	15.04	5.90	1.40
Hydrochloric acid cooked curd	8	–	9.44	0.18	4.71	15.03	5.20	1.20
Commercial caseins								
Grain-curd	7	2	6.84	0.50	2.78	14.71	8.90	
Self-sour or lactic	12	–	7.70	0.88	3.46	14.38	9.70	
Argentine (self-sour)	8	–	8.20	1.10	3.81	14.66	11.50	
Sulfuric acid	23	–	7.97	1.41	3.92	14.42	10.40	
Hydrochloric acid	15	–	7.37	0.56	4.52	14.39	8.70	
Acid cooked curd	18	–	8.16	0.65	4.45	14.40	7.50	
Rennet	3	–	8.29	0.63	7.97	14.41	7.90	
Buttermilk (ejector method)	3	–	8.41	6.65	1.62	14.78	9.23	

* F. L. Browne, J. Ind. & Eng. Chem., 13 (1919), 1019—Averages taken from tables.

lactic acid remaining in contact with the curd for a considerable time and the breaking up of this curd into granular condition with the ejector method of heating, provides conditions favorable for thorough washing. The adaptability of this curd to washing is shown in Table III. The caseins from the washed curds showed an average ash content of 1.78 per cent compared to an average of 2.46 per cent for the unwashed curd, while the "acidity" of the washed curd was 8.06 per cent compared to 9.02 per cent for the unwashed. The ejector, self-sour method casein is generally considered superior to that made with the usual sulfuric and hydrochloric (muriatic) acid pressed or cooked curd methods. It dissolves more readily, has better strength, and retains a fluid, viscous

body at room temperature, when dissolved in the usual quantity of water and solvents. If the curd is not well washed, it will not show up so well but with very little washing the casein will have a lower ash content and better properties than the self-sour, vat method casein.

The self-sour casein made by the vat method has in general a medium ash and viscosity and possesses most satisfactory properties as evidenced by the general preference for this type of product. The "acidity" may be rather high as the curd secured with the vat method, when a drain rack is not used, does not favor easy washing. It may be well washed by breaking the curd up finer in the wash water, but this is not usually done.

Sulfuric and Hydrochloric (Muriatic) Acid Pressed and Cooked Curd Caseins.

Casein made by the usual method of precipitating with either sulfuric or hydrochloric (muriatic) acid at a temperature of 120° to 125° F. (48.89° to 54.44° C.) has a high ash and viscosity, the hydrochloric acid product having much the higher viscosity of the two. The chunky curd secured by these methods does not favor the reduction of the ash content by washing. Table III shows that washing the curd did not reduce the ash content, but did materially reduce the "acidity" and for this reason should be practiced. There was a reduction in "acidity" with both the sulfuric and hydrochloric (muriatic) curds but no reduction in the ash content as shown by the average analyses. Using more acid than is required for securing a clear separation will injure the quality of the casein due to the additional acid enmeshed in the curd, which increases the viscosity and decreases the solubility.

The high temperature used in making cooked curd further increases the viscosity, and in case of the hydrochloric acid may be quite objectionable. The cooked curd caseins have a much lower "acidity" due to the hot water being so well intermixed with the curd during the cooking process and its later draining away. The sulfuric and hydrochloric (muriatic) pressed and cooked curd caseins have a heavy, viscous body which retards somewhat their going into complete solution with the standard methods of dissolving. Upon cooling the solutions have a decided tendency to gel, more especially the cooked curd casein.

Grain-Curd Casein.

The lower precipitating temperature used with the grain-curd method allows of obtaining a granular curd which can be readily and thoroughly washed, and eliminates the influence of heating the curd. The analyses of commercial grain-curd caseins given in Table III show what a low ash casein may be secured. Those caseins, the curd of which was

washed twice, have an average ash content of 2.78 per cent, which is only about half of what it would be if precipitation had been carried out with hydrochloric (muriatic) acid at a temperature of 120° F. (48.89° C.). It is possible to secure a still lower ash by means of additional washing. Typical grain-curd casein compares very favorably with a good grade of self-sour or lactic casein, being readily soluble and possessing the desired physical properties. When properly made it does not have the heavy viscosity of the usual acid pressed and cooked curd caseins.

Rennet Casein.

Properly made rennet casein has an extremely high ash content and viscosity. The ash content is a good criterion of the quality of casein and is generally used, in connection with the fat content, for a standard. In case the skim-milk has developed any appreciable acidity before it is made into rennet casein the ash content will be reduced, making it much less desirable for casein plastics, for which it is so largely used. The thorough washing which rennet casein should receive has no effect in reducing the ash content.

Rennet casein is not desirable for the uses for which acid casein is intended, because of the greater difficulty of dissolving, and its less desirable physical properties.

Buttermilk Casein.

The composition and properties of buttermilk casein show a wide variation, depending largely upon the buttermilk from which it is made. The fat content is invariably much higher than skim-milk casein and may have quite an injurious effect upon the quality of the product, especially in connection with some of the other influencing factors. Drying a high fat or unwashed curd at a high temperature lowers the quality of the resulting casein.[7] Buttermilk caseins in general have a low viscosity, giving a rather thin solution when dissolved in the quantity of water and solvents generally used with other caseins. Commercial buttermilk caseins are likely to contain considerable insoluble material.

Pure Casein

A method for making pure casein, claimed to be superior to the product made by the method originally devised by Hammarsten, has been developed by Van Slyke and Baker.[8] The work of Van Slyke and Bosworth, which showed that soon after milk coagulates in the ordinary process of souring, all of the calcium and inorganic phosphorus is in solution, suggested the possibility of preparing a pure casein that would not be subject to the objectionable conditions connected with the Hammar-

sten method. Their supposition was that if an acid could be introduced into undiluted skim-milk in a manner resembling the natural souring of milk, the objectionable features could be reduced and a pure product obtained. By gradual addition of the acid below the surface of the skim-milk and simultaneous high-speed mechanical stirring the most important condition of natural souring is approximated in their method.

Apparatus Required.

The apparatus used for coagulating the casein in one liter of skim-milk, rapidly stirred, while slowly adding acid under the surface, consists of four parts:

1. A relatively low form of open container, such as a wide-mouthed bottle, precipitating jar, or a porcelain enamelled container, in which the slow coagulation takes place.
2. An ordinarily graduated burette for holding the acid, with stop cock and holder.
3. A capillary glass tube with a 1.5 to 2 mm. diameter bore and a specially made delivery tip. Bending the tip up somewhat and flattening and widening the opening in the form of a narrow slit prevents milk or particles of casein from being forced into the delivery tube and interfering with the flow of acid. The other end of the tube is fastened to the tip of the burette with close fitting connections of rubber tubing.
4. A mechanical stirring-rod so designed that it will maintain a rapid, uniform speed and stir the skim-milk thoroughly without producing excessive foam. The stirring rod they used was made of a piece of perfectly straight glass tubing of about 1 cm. diameter, mounted on two annular ball bearings, attached to the glass rod by interposing sections of rubber tubing of suitable thickness. One bearing was in the middle of the glass tube just above the coagulating vessel and the other at the upper end just below the point where the rod was connected to the motor shaft by means of rubber tubing.

The stirrer is placed in the vessel at one side of the center to avoid the foam which would result from the whirlpool formed if it were in the center. The delivery tip of the submerged tube is so placed in relation to the propeller blade that the whirling skim-milk washes the end of the tip but is not thrown directly against the opening in the tip, which might force milk or particles of casein into the tube and clog it.

Acid Used.

While they state that many acids can be used, their preference after working with acetic, lactic and hydrochloric, was either normal lactic

acid or a mixture consisting of one part of normal hydrochloric acid and one or two parts of normal acetic acid. Acetic acid did not always give satisfatory coagulation of the casein in undiluted milk. Hydrochloric acid alone gave satisfactory results and the only objection to its use was the danger of adding too much acid.

Coagulation of the Casein.

The coagulation of the fresh, undiluted skim-milk by the addition of acid is described as follows:

"To 1 liter of milk, normal acid is added in the apparatus described above, the stirrer rotating as rapidly as possible without causing excessive foaming, which is usually at the rate of 2,000 to 3,000 revolutions per minute. The acid is allowed to flow into the milk at a rate which will not cause appreciable coagulation as shown by application of the following sedimentation test.

"A few cc. of the milk are whirled in a sedimentation tube for a short time. If, on pouring the liquid from the tube, a sediment remains at the bottom, it is an indication that acid has been added too rapidly and has caused some coagulation of casein at the point of contact of acid and milk. Such action can also be avoided by having the milk and acid at low temperatures. Generally speaking, the first 45 cc. can be added in 30 minutes. The addition of acid is continued until the amount reaches about 60 cc., after which the rate is decreased somewhat until the coagulation point is nearly reached, as shown by the tests given below; and then, before addition of more acid, the mixture is allowed to stand about 3 hours, the rate of stirring being diminished to 500 revolutions per minute or less. The amount of acid to be added above 45 cc. can be ascertained by taking a sample of the original milk and titrating to the coagulation point, and then adding this amount of acid in the manner above indicated. The use of some brom-cresol purple as indicator aids in detecting the approach to the coagulation point. This can be more accurately ascertained by diluting a few cc. of the acidified milk with an equal volume of distilled water and centrifuging in a sedimentation test-tube, when the casein completely separates. This is due to the fact that the hydrogen ion concentration of acidified milk is increased by dilution and that this increase is sufficient, in case of milk near the point of casein coagulation, to throw down the casein. When this test shows no casein separation, then addition of acid is continued and the test repeated at intervals until such separation of casein occurs.

"During the latter stages of addition of acid, foaming may make some trouble. This, however, can be obviated or controlled by adding a few drops of octyl alcohol, or by using a stirrer free from vibration, or by having the vessel that contains the milk about double the capacity re-

quired to hold the amount of milk used, which will usually provide sufficient room to accommodate the foam; any foam forming subsides promptly when the coagulation of the casein takes place.

"After standing under gentle stirring for 3 hours with acidity just below the point of casein coagulation, addition of acid is continued slowly, accompanied as before by rapid stirring in order to obtain the particles of casein coagulum in the finest possible state of division. Addition of acid is now continued to the point at which a portion of the mixture, when centrifuged, gives a definite supernatant layer of solution above the casein. Ordinarily, it requires about 90 cc. of normal lactic acid or of a mixture of 1 part of normal hydrochloric acid and 2 parts of normal acetic acid to accomplish this for each liter of milk used. Less normal hydrochloric acid (about 75 cc.) is required. When the coagulation is complete, as shown by the test, the mixture is allowed to stand 2 to 4 hours with gentle stirring.

Treatment of Coagulated Casein.

The operations following the coagulation of the casein, including new features,[9] are as follows:

"The separated casein is washed with successive portions of water in a centrifuge, until the wash water begins to show slight cloudiness, indicating that the minute particles of casein are going back into suspension and do not, therefore, separate completely in the centrifuge. The casein precipitate still contains some phosphates of calcium and magnesium in colloidal condition, and these compounds can be completely removed only by special treatment. When the washing with water is carried to the final point, indicated by cloudiness of the supernatant wash water, the centrifuged casein mass is beaten back into suspension with water by the stirrer. Then sodium hydroxide of about tenth normal concentration is added until the resulting solution has a neutral reaction indicated by hydrogen ion concentration of pH 7. In this solution of sodium caseinate, the calcium and magnesium phosphates remain in suspension and can be mostly removed by centrifuging. However, enough remains in suspension to give the centrifuged solution a slight opalescence.

"The solution of sodium caseinate is diluted so that it contains the equivalent of 1 to 1.5 per cent of casein. This is then precipitated by the same treatment used in the case of the original skimmed milk, except that it is found preferable to add only normal acetic acid, without hydrochloric, to a reaction of pH 4.7. The solution obtained after centrifuging the precipitate is water clear. The casein is finally suspended in water and dilute acetic acid added to the reaction of pH 4.7. In this suspension, the final trace of phosphates is removed by electroly-

sis, the suspension being placed in the middle compartment of a three-compartment cell, separated by sheets of collodion. Bacterial action is prevented by toluene. After this treatment is completed, the casein is centrifuged with water and treated, in succession, first with hot, neutral 70 to 80 per cent alcohol, then with absolute alcohol, absolute ether, and finally with petroleum ether of high boiling point, or with benzene or toluene. According to the method formerly used in our laboratory, ether was the final reagent used in making the preparation of casein. It was found that, in spite of precautions, the reduced temperature caused by the rapid evaporation of the ether resulted in condensation of moisture from the air about the particles on the surface of the casein mass. The effect of this condensation is to convert the fine particles of the exposed surface into a sticky, gum-like crust, which can be made sufficiently fine only by grinding. However, if treatment with ether is promptly followed by treatment with some volatile liquid of higher boiling point, the evaporation is slower, there is no condensation of moisture, and the particles of casein retain their extreme fineness.

"The reason for treatment with hot 70 to 80 per cent alcohol is the removal of a protein, to which Osborne recently called attention, which we find, so far as it has been studied, possesses properties distinguishing it markedly from casein."

Ash and Phosphorus of the Casein.

"The ash and phosphorus content of casein prepared in the manner described depends upon certain details in the method of preparation. Holding the casein in the uncoagulated condition at a degree of acidity just below the point of coagulation results in a preparation low in phosphorus, the minimum amount being 0.80 per cent. This result is due to the fact that the suspended inorganic phosphate ($CaHPO_4$) in milk is completely dissolved by the treatment. It requires, in our experience, 3 hours at least to make sure of complete solution of the insoluble phosphate. It is, however, not advisable to let the casein stand in this uncoagulated condition more than 6 hours, especially if not kept at a temperature of 18° C. or lower, because some slight hydrolysis of the casein may occur, which tends to affect unfavorably the properties of the preparation.

"If casein after coagulation is held in suspension at a hydrogen ion concentration above the isoelectric point, the amount of ash is low, ranging from 0.05 to 0.15 per cent. This result is due to the decomposition of the calcium caseinate, the acid combining with the calcium and leaving the casein base-free. Complete action requires 2 to 4 hours. Longer standing under these conditions is not likely to cause any hydrolysis, according to our experience. When, therefore, it is desirable for con-

venience to carry the preparation over night and complete it the second day, the casein can remain in this completely coagulated condition without affecting its character.

"When the insoluble inorganic phosphate is not completely dissolved before coagulation, it does not appear possible to remove it completely after coagulation, no matter how long the mixture is allowed to stand. This is indicated by the results embodied in the following table:

Time of Standing before Coagulation	Time of Standing after Coagulation	Ash	Phosphorus *
Hours	Hours	Per cent	Per cent
0	6	0.15	0.85
0	24	0.14	0.86
0	24	0.15	0.83
3	0	0.46	0.81
4	0	0.43	0.81
1	12	0.15	0.85
3	15	0.05	0.80
3	18	0.11	0.81
4	4	0.10	0.81
16	4	0.10	0.81
30	10	0.10	0.80

* We are indebted to Mr. R. F. Keeler for repeating and confirming these determinations.

"The first three series of results in the table show that when the mixture is not allowed to stand awhile in the uncoagulated condition at a point of acidity just short of coagulation, the phosphorus content is relatively high. The cases in which the mixture was allowed to stand 3 and 4 hours before coagulation, but not at all after, show high ash but low phosphorus content, indicating removal of inorganic phosphorus and retention of calcium in the form of calcium caseinate. The last five series of results in the table, from preparations obtained by allowing the mixture to stand 3 to 30 hours before coagulation and 4 to 18 hours after, show a minimum of both ash and phosphorus. In no case was it found possible to obtain a preparation with a percentage of phosphorus lower than 0.80 by prolongation of the period of standing."

Advantages of Method for Making Pure Casein.

Advantages in favor of the method over other methods of preparing pure casein to which attention is called are:

1. "A preparation of casein can be made within 10 hours. Other methods usually require 2 or 3 days.
2. "Uses the skim-milk without dilution and saves much time and labor in lessening the amount of material to be handled.

3. "Unnecessary excess of acid is avoided. The hydrogen ion concentration of the coagulation mixture is only slightly above the isoelectric point of casein; that is, about pH 4.5 to 4.6.
4. "The contact with reagents need not exceed 6 to 8 hours.
5. "The dried casein obtained forms a very fine powder even without grinding; usually about 90 per cent will pass a screen containing 100 meshes to the inch.
6. "This casein very easily and quickly dissolves to a water-clear solution in dilute mono-basic alkali solution and also in excess of lime-water. Lime-water solutions of the casein, when neutral, are opalescent.
7. "It is possible to obtain without appreciable loss all the casein in the milk in pure form. In other methods the losses of casein in manipulation are considerable and unavoidable."

Edible Casein

Available information on the type and method of manufacturing edible casein used in the preparation of food products for human consumption is rather meager. Needless to say, casein for this purpose should be made in a most sanitary manner, to insure its cleanliness and purity.

Edible casein has been quite generally regarded as being made by the rennet method, which has the advantage of more favorable sanitary control on a basis of commercial manufacture, but other methods may also be used.

The method for the preparation of pure casein developed by Van Slyke and Baker given in this chapter will give an exceptionally good quality of edible casein when made under proper sanitary conditions. The self-sour ejector method will also give an edible casein of good quality when made with proper care. In using this method for making edible casein, the precipitated curd should be handled in a manner which will give conditions most favorable for washing out the free lactic acid. Agitating the firmly coagulated skim-milk before heating it with the ejector will give a much finer curd, more favorable for thorough washing. By washing the fine curd repeatedly, a good quality of edible casein may be obtained.

The use of hydrochloric acid in making commercial casein leaves impurities in the product, which are detrimental to health, unless removed. These impurities can be removed by a process of redissolving, washing, and reprecipitating. This method is now used by one manufacturer in producing "Nucleo," which will be found described in the chapter on "Medicinals."

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Chapter 4

Casein in Paints

By Albin H. Warth

Paint may be defined as a solid material, usually a pigment, pulverized and intimately mixed with a liquid, called the vehicle, to a fluid mass which may be applied by a brush or spray to form a uniform coating for the purpose of protecting or beautifying the surface to be coated. Both the solid and liquid portions may consist of single materials, or of combinations of materials.

Classes of Paints.

Paints may be divided into two broad classes: water paints and oil paints. While in general principle both classes are fundamentally the same in their structural make-up, yet they differ in certain important respects. In both the function of the pigment is to cover the surface, and the purpose of the vehicle is to make it possible to apply the pigment successfully. However, the inert pigment, or pigments which are used for thickening rather than for covering in the case of oil paint may become the covering pigments when used in water paint. In oil paints the vehicle is a drying, or semi-drying oil with a thinner, such as turpentine or naphtha; in water paints the vehicle is generally water only.

Oil paints are marketed in two principal forms: (1) a semi-paste to which the consumer adds the thinner, and (2) a liquid paint ready for use. Corresponding to these in the case of water paints are (1) the dry powder, to which the user adds water for a thinner as well as for a solvent for the binder, and (2) a ready-mixed, liquid paint. Oil paints are sold chiefly in the liquid form, and water paints as dry powders. The disadvantages of marketing water paint in the liquid state are in the larger sized containers required, extra handling, increased cost for freight, and danger of rusting cans. No advantage is gained by the consumer, who can buy water as cheaply as the paint manufacturer.

Types of Pigment.

The pigments used in paints may be divided into three classes: (1) Covering pigments, (2) Thickening pigments, and (3) Coloring pig-

ments. Except where the coloring pigment functions as the covering pigment, the covering pigments which are mixed with oils and varnishes are one or more of the following: lead carbonate, zinc oxide, lithopone (zinc sulfide on barytes), titanox (titanium oxide on barytes), and timanox (antimony oxide). In water paints all of these are included in the list of covering pigments, while in addition practically any white, pulverized earth, as whiting, clay, magnesium silicate, talc, etc., will cover solidly. These latter pigments would all be more or less transparent in oil paint, yet they find a very extensive use in water paints, because with water they are opaque, and they are obtainable at one-tenth the cost of the covering pigments for oil paints. The index of refraction between the white pulverized earth and water is sufficiently large to produce the required opacity and hiding power, which is not the case with oil.

Typical thickening pigments are asbestine, whiting and clay. These are used in oil paints principally as agents to suspend the covering pigments in the vehicle, and to facilitate the brushing out of the paint. In water paints asbestine is used as a suspending medium, and clay as an aid to brushing, while whiting is usually the chief covering pigment.

Coloring pigments of the same general class are used in both oil and water paints. Those which are most extensively used in the manufacture of water paints will be referred to later.

The Vehicle.

The vehicle in paint functions both as a binder and as a brushing medium. It is composed of three principal parts: binder, drier or insolubilizer, and thinner. In oil paints the principal binders are fixed drying oils, such as linseed, tung, soya, etc., while in water paints the principal binders are solutions of glue, casein, dextrine, and silicate of soda. Considered from the standpoint of the cost per pound for ingredients, glue, casein, linseed and tung oil are not far apart in value, yet the binder cost per gallon in oil paint is about five times the cost in water paint because of the larger proportion of binder used.

Driers.

In oil paints the driers are metallic salts, such as lead oxide, manganese borate, cobalt linoleate, etc., which oxidize and polymerize the oil. In water paint so-called "insolubilizers" are used, principally in connection with glue and casein. These make the paint damp-proof, and to a certain extent waterproof, depending upon the composition of the paint. When glue is used as a binder formaldehyde is generally the insolubilizer employed, and it is brushed over the surface of the dry paint. With a

casein binder the insolubilizer consists of lime mixed in with the rest of the materials composing the paint.

Thinners.

The correct proportions of binder and pigment would, in most cases, make a stiff, putty-like mass; therefore a thinner must be used to give it sufficient fluidity so that it may be applied by spraying or brushing. In oil paints the thinners used are turpentine, turpentine substitutes such as petroleum distillates having a distillation range approximating that of turpentine, or coal tar naphtha. In water paint water is used.

Historical.

M. D. Swartz * states that translations from ancient Hebrew texts mention the decorator or painter of the type still existing in a number of European countries, principally in Austria, Hungary, Jugo-Slavia, and the Balkans and Turkey. These texts admonish the housewife to save the curd for the autumn visit of the painter. They instruct the husband, the home owner, to go forth into the hills and bring back the color earths. They admonish the family to have the materials ready to call in the decorator to paint the house for the Succoth, which is the ancient Hebrew Thanksgiving holiday.

This same system of painting still exists in many villages in the European countries mentioned, with the exception that now the painter supplies the earth pigments, and the housewife supplies the curd. In these countries most of the houses are of stone or brick, with roofs of thatch, slate or tile. The interior walls, which are practically all plastered, receive the painting, while the exterior requires little or no decoration. In the rural towns of Hungary, when painting is necessary, the housewife calls on the painter, makes an appointment for the job, saves enough skimmed milk, and prepares sufficient "klabber" or curd for his work. She picks out the colors wanted, which are selected from ochres, umbers, siennas, and red oxides. The painter brings the colors and sufficient freshly burned lime, mixes them on the job, and goes to work.

The art of using casein in oil paints dates back to the most renowned schools of interior decoration. Milk was permitted to sour and then mixed in with the pigment and linseed oil, and used to produce high light effects in portraits in mural decorations. Michael Angelo is said to have used a combination of sour milk, oil and pigments for such a purpose.

* M. D. Swartz of the Farboil Paint Products Co., Baltimore, Md., has materially assisted the writer in securing information concerning the history and certain other phases of the cold water paint industry, and this assistance is hereby gratefully acknowledged.

In the United States the first extensive commercial use of casein in paint dates back to the early part of the nineteenth century, although its use in Germany and England had already been highly developed at a much earlier period. Painting with cold water paint of some description was known in the early days of our colonization when such paints were applied to the wood and stonework of the dwellings, fences and barns. The early settlers knew that burned lime made into whitewash, while pure and sanitary, flaked and powdered from the surface, and its durability was very limited unless it were mixed with salt and soured skim-milk.

Statistics.

There are no accurate statistics available but it is estimated that over 150,000,000 pounds of water paints, including calcimines, are produced annually in the United States. Of this total at least 15,000,000 pounds are casein paints. These have an estimated value of nearly $1,000,000, while the estimated value of the whole output of both washable and non-washable water paints is approximately $5,000,000.

The United States is the leading producer of cold water paints, although England and Germany produce large amounts of the higher qualities, including some linseed oil casein paints. It is difficult to get estimates of European production.

The annual consumption of casein by the paint industry is estimated at 800,000 pounds. While this quantity is small when compared with that consumed in other lines of industry, yet it is sizable, and the amount will increase greatly with the growing demand for washable cold water paints, and the further application of the spray brush for exterior painting.

Distribution of the Industry.

The cold water paint industry extends through thirteen states, including New York, Ohio, Pennsylvania, New Jersey, Massachusetts, Michigan, Indiana, Illinois, Washington, California, Missouri, Maryland, and Tennessee. The largest exclusive producers of cold water paints are located on the Eastern Seaboard, in the Middle West, and on the Pacific Coast.

In 1925 there were 48 firms in the United States listed as manufacturing cold water paint. Many of these firms make both calcimines and exterior cold water paints, while some produce specialties such as fire retardant, stucco, and insulation paints. Paints are not often marketed as "hot water," "cold water," or "washable," but are sold under trade names, which are extensively advertised.

Demand for Water Paint.

The ease of application and the low cost of a paint which is miscible with water, as contrasted with the greater care required in the application

of an oil paint prepared from more expensive ingredients, have stimulated both the development of, and the demand for, a paint which can be widely used for both exterior and interior purposes. Such a paint, which is washable and waterproof to a considerable degree, is known as cold water paint. To-day there is a tremendous field for its application to exterior shafts, gasoline stations, etc., and especially to stucco or cement surfaces which are difficult to paint effectively with oil paint.

Classification of Water Paints.

Water paints may be divided into four principal classes:

1. Distempers and Calcimines.
2. Interior or Exterior Washable Paints.
3. Water Paint Enamels.
4. Art Colors.

Of these the exterior or washable cold water paints are by far the most important from the standpoint of the use of casein. Calcimines largely employ glue or dextrine as a binder. Water paints containing linseed oil or other vegetable oils are still in a state of experimental development, but judging from the recent progress made in Europe they may eventually become of considerable commercial importance. Enamel paints are in paste form ready for mixing with water. The art colors are generally made from lump pigments ground in glue water.

Distempers.

Distempers are usually mixtures of pigments with glue as a binder and water as a thinner. Distempers employing inexpensive pigments are applied to plaster, stucco, brick and stone; they serve fairly well in dry buildings, but they are not satisfactory for exterior work, except where permanence is not required. Buildings at expositions, amusement parks, etc., are often painted with distempers in which whiting is colored with cheap, dark colored pigments. The pigments are made into a pulp with water, the color being mixed separately from the white, and then the white and color are blended. A small amount of alum is sometimes added, which serves to harden the glue on drying and make it more damp resisting. The term "distemper" is broadly applied, especially in England, to cover calcimines and other cold water paints; water colors and fresco colors also belong to this class. Where casein is used as a binder it requires the addition of 15 to 20 per cent of borax, based on the weight of the casein, as a solvent.

Calcimines.

With the advent of the plastered wall and the growing demand for more artistic interior decoration, water paints were prepared which were

relatively free from the caustic properties of whitewash, and which could be tinted to any desired shade. These had for their base the pigment known as whiting, which is washed chalk, and became known to the trade as calcimines. They are simply distempers, made and sold in the form of dry powders. Often a wall size is used to seal the pores in the wall and make a better foundation on which to apply the calcimine.

Calcimines are also frequently called Kalsomines, and not incorrectly, although the former term is to be preferred on account of its being directly derived from calcium, which is the real elemental base of all calcimines. Modern products of this class are made from the finest washed and floated whiting, combined usually with hide stock glue. They are easily miscible in cold water, and are used for their soft, velvety, water color effect. They can be applied to plastered walls, wood, or wall board, but their use must be confined to interior decorating and they are not washable as are casein cold water paints.

Washable Paints.

Interior or exterior washable paints, generally speaking, are casein paints, since they are cold water paints in which casein is used as a binding agent for the pigment. It is with this class of paints that we are particularly concerned, since the future of the industry lies in the development of high quality washable paints, in which casein is the important ingredient, rather than of the cheap forms of calcimines, which do not employ it, and which are little superior to whitewashes.

Water Paint Enamels.

Water paint enamels are water paints which leave a hard, glossy, enamel finish upon evaporation of the vehicle constituent. They are made by grinding white and colored pigments with a mixture of silicate of soda as a binder and water as a thinner, and are always sold in liquid form. The following formula for pea green enamel is characteristic of the composition of such enamels:

Whiting	9 lbs.
Lime proof green	1 lb.
Silicate of soda (32° Bé)	2 lbs.
Water to make one gallon.	

Products of this class are difficult to keep in good condition and for this reason have not been exploited commercially to any great extent.

Art Colors.

Art colors or water colors are colored pigments ground in glue water and a little glycerine and sold in semi-paste form in collapsible tubes, and also in small blocks, made by evaporating water from the semi-paste.

The pigments most frequently used for this purpose are zinc white, Naples yellow, pale ochre, cinnabar, madder lake, burnt ochre, burnt sienna, cobalt blue, ultramarine, Prussian blue, chrome green and ivory black. A pigment to be suitable for artists' use must be ground as finely as possible and sometimes very carefully levigated and dried, so as to get a soft powder of good bulking capacity, which will work smoothly with maulstick and palette, and tint satisfactorily when applied with a brush.

Composition of Cold Water Paints.

Cold water paints are composed essentially of:

1. A pigment for covering.
2. A filler to prevent settling and make the paint work easily.
3. Dry colors for tinting.
4. A binder.
5. An insolubilizer.

The best pigment, and the one having the maximum covering power per pound of material, is the highest grade of imported, washed chalk whiting. This will be referred to later.

Clay and asbestine are the most satisfactory fillers, either giving equally good results.

The dry colors used are chrome greens and yellows and earth colors such as ochres, umbers, and siennas. But few coal tar dry colors can be used for tinting as many are not sufficiently fast to alkalies. Those which are used are referred to as "anti-calc" colors, indicating that they are proof against the alkaline reaction of lime.

The purpose of the binder is to hold the pigment and color tightly to the surface which is to be painted. Substances used for this purpose include casein, glue, dextrine, water glass, and water soluble gums such as Arabic, Karaya, Indian, etc. Casein needs to be treated with an alkaline solvent such as borax, trisodium phosphate, sodium borophosphate, potassium oxalate or ammonia before it can act as a binder, while the other four classes of materials are soluble in water. Glue requires hot water, while a temperature above 60° F. is satisfactory for the other binders.

An insolubilizer, to render the paint washable, is necessary when casein is the binder employed. The substances used for this purpose are lime, hexamethylenetetramine and aldehyde ammonia.

Chalk Whiting.

The chalk whiting, which is so extensively used in the paint industry, is made from lump chalk which occurs in natural form in the chalk cliffs of Dover, England. It is ground, levigated and dried, and is marketed

in several grades. The finest is known as Paris White and is used in the best water paints. This white is so finely ground that 99.7 per cent will pass through a 300-mesh sieve. A medium grade is known as gilders' whiting and is used in the picture frame and gilders' crafts, while a third, and the coarsest grade, is used in putty making.

Chalk is natural, amorphous, calcium carbonate, composed of the delicate skeletons of rhizopods and other marine organisms. It is found in Alabama, Iowa and Nebraska, but most of the chalk of commerce is imported from England. Precipitated calcium carbonate, either specially prepared or obtained as a by-product in the making of lye, cannot be advantageously used for making high quality water paints. Precipitated calcium carbonate has a crystalline structure, minutely crystalline to be sure, but sufficiently so to lower the index of refraction, and cause poor opacity in the paint made with it. It also has other physical properties not suited to paint making.

Hydrated Lime.

As one of the most important constituents of casein paint is commercial hydrated lime, and as the hydrated lime offered in the market is not infrequently of a very uncertain quality, it should be purchased under specifications which are sufficiently definite to insure receiving material that is carefully prepared and uniform in quality.

The material purchased for casein paints should be prepared by slaking fresh, well burned quicklime with about a third of its weight of water. It should be a white, amorphous powder, sufficiently fine to pass a 100-mesh screen, and should be packed in moisture proofed lime barrels, or air tight containers, to prevent its deterioration during transportation or storage. The sum of the calcium carbonate and magnesia must be less than 3 per cent, and the iron oxide must not exceed 0.25 per cent.

The following is an analysis of a good grade of commercial hydrated lime, produced by slaking lump lime and then bolting through cloth:

	Per cent
Silica	1.40
Iron oxide	0.23
Alumina	0.77
Total lime	71.29
Magnesia	1.56
Loss on ignition	23.40
Moisture	0.50
Sulfur	0.76
Active lime	66.49
Carbon dioxide	0.95
Calcium carbonate	2.16
Fineness—200 mesh	91.6
Fineness—100 mesh	100.0

An analysis of a Virginia hydrated lime marketed in paper sacks, is as follows:

	Per cent
Silica	0.60
Oxides of iron and alumina	0.44
Calcium oxide	71.89
Magnesia	0.75
Carbon dioxide	1.08
Sulfuric anhydride	0.21
Combined and hygroscopic water	25.02

White Pigments.

The natural white pigments which are used in water paints are whiting, alabaster, magnesia, barytes, china clay, and asbestine. Artificial pigments such as titanox, zinc white and lithopone are not used to any great extent, though they are necessary in the preparation of specialty cold water paints which contain linseed or other drying oil. Titanox consists of titanium oxide precipitated on barium sulfate, and contains at least 20 per cent of titanium oxide. As it is a comparatively new pigment its use has not been exploited in cold water paints. Zinc white, which is zinc oxide, is obtainable in different grades; the Red Seal, or equivalent quality, is quite satsfactory for paints. Lithopone is a chemically precipitated pigment containing about 70 per cent of barium sulfate, and 30 per cent of zinc sulfide. It is also known as albalith, ponolith, Beckton white, etc. Like titanox it is sometimes used with zinc white, or it may be used alone.

Colored Pigments.

The colored pigments employed in the water paint industry include all the earth colors, a number of artificially prepared mineral pigments, and a few coal tar lakes. For reds there are Tuscan red, Indian red, Canadian red, American vermilion, and Venetian red; also coal tar lakes such as para red and toluidene red, the para red being used more extensively. The red earth colors are of many different hues and strengths. Venetian red has the lowest ferric oxide content, and Canadian red the highest.

Among the blues there are ultramarine blue, the iron blues, such as Chinese blue and Prussian blue, and turquoise blue which is a coal tar blue of remarkable beauty for tinting, being free from the reddish undertone of ultramarine, and the reddish and greenish undertones of the iron blues. Indigo blue may be used to great advantage as it is no longer regarded as a very expensive color. Cobalt blue, or smalts, is sky blue in color and has a limited use in water paints.

Among the yellows there are chrome yellow, which comes in both lemon and deep yellow shades, chrome orange, and zinc yellow, all of

which are chromates. Naples yellow, which is lead antimoniate, and cadmium yellow, or cadmium sulfide, are also used, the latter being a splendid working and permanent color for lemon shades. The ochres, however, find the widest application because of their low cost. They vary greatly in shade and composition from a pure yellow to a dull orange or red. The French ochres are considered the best as they are the most carefully prepared. They contain about 19 per cent of ferric oxide, the remainder being silica and alumina.

For browns there are manganese brown and the ferric oxide colors such as raw and burnt umbers, and raw and burnt siennas. The Italian siennas are very deep in tone, and contain about 70 per cent of ferric oxide. The umbers contain but 7 to 14 per cent, and some manganese dioxide, which gives them their peculiar hue.

In blacks there are carbon gas blacks of many varying qualities. What is known as carbolene black is generally a superior black. Ivory black is used only to a limited extent on account of its cost.

Type of Binder.

Although a washable water paint may be made in a number of ways,—with a combination of glue and paraform, with silicate of soda and starch, with albumin and aldehyde,—it may be made most simply, quickly and cheaply by using casein as a binder. This is one of the chief advantages obtained by using casein.

The various grades of glue do not possess the waterproofness of casein, even under the most effective waterproofing treatment. Since casein is more readily rendered insoluble than glue, more care is required in the way the insolubilizer is incorporated, lest the reaction take place too rapidly and the casein become insoluble sooner than is desired. There is not, however, the danger of the casein binding medium of the paint becoming as brittle as a glue medium where an excess of the insolubilizer has been added.

Casein does not have the same power to prevent settling as glue, although the buoyant effect of the latter will naturally vary to a considerable extent according to the grade of glue, and the temperature of the water employed. When a paint with a casein binder has a tendency to thin out and settle when water is added, it becomes necessary to hold the materials in suspension by adding something besides clay. This is usually accomplished by incorporating in the paint 2 per cent of cold water soluble starch. Starch, however, possesses a disadvantage in washable paints as there is no way in which it can be rendered insoluble after the paint has been applied.

Quality of Casein Used.

In the manufacture of cold water paints preference has been given to the caseins of domestic manufacture over those of foreign make. Most of the latter are lactic acid precipitated, and, generally speaking, such caseins have a lower absorptive power than either the hydrochloric or sulfuric acid precipitated caseins. Sulfuric acid casein is the type usually preferred for the manufacture of water paints. Argentine caseins often contain dirt specks which are apt to discolor the finished product, especially in the case of whites. However, there are superior grades of lactic casein now being produced in Argentina.

Casein for use in paints must be finely ground and bolted. Those which are compounded with alkali to make them soluble are marketed in a more or less flour-like form, or very considerably finer than 100 mesh.

Specifications for Casein.

Casein for cold water paints should be purchased under specifications which will insure satisfactory uniformity in the material delivered. It should be clean, free from rancid or sour odor, and completely soluble in a solution containing 15 per cent of borax on the weight of the casein. The viscosity of a 10 per cent solution, using 15 per cent of borax as a solvent, should not vary appreciably from that of the type sample.

The casein should be ground to such a fineness that practically all of it will pass through a 100-mesh sieve, and it should have the following chemical characteristics:

The moisture content must not exceed 9.0 per cent.
The ash content must not be over 4.0 per cent.
It must not contain more than 1.0 per cent of fat.

The Insolubilizer.

In the manufacture of an outside water paint the binder must be one which will go into solution readily in cold water and which will become insoluble on drying. Casein is the best binder for the purpose but it requires the addition of an insolubilizer to render the paint waterproof.

Hexamethylenetetramine is regarded by the manufacturers as being too costly for this purpose and consequently most of the casein cold water paints on the market have lime as the insolubilizing agent. The action of hydrated lime in the paint is to dissolve the casein when water is added, and then form insoluble calcium caseinate on drying. Too little lime will not produce the amount of calcium caseinate necessary to make the paint waterproof and it will not be washable. An excess of lime will prevent the casein from going into solution when the paint is mixed with

water, and there is a tendency for the paint to dry hard and brittle and flake off. While hydrated lime is the best solvent for this purpose its use must be very carefully regulated if satisfactory results are to be obtained.

The principle involved in the use of aldehydes as insolubilizers is that they react chemically with gelatin, casein, and albumin to form addition products which are insoluble in water. This reaction is perhaps most marked with albumin, next with casein and least with gelatin. The reaction between paraform, which is the polymer of formaldehyde, and gelatin is easily controlled, but this is not the case with casein. In fact, the speed of reaction of formaldehyde or paraform with casein is so great that it is almost impossible to regulate it, and precipitation takes place almost as soon as these substances are mixed. This makes it necessary to use the formaldehyde in a modified form so that its reaction with casein may be retarded. Ammonia acts as a retardant as it combines chemically with formaldehyde, producing a condensation product known as hexamethylenetetramine, or hexamine, $(CH_2)_6N_4$, which is a very effective agent for controlling the insolubilization of casein. Its action is further retarded by ammonia or mild alkalies, and accelerated by practically any substance of an acid nature or by means of heat. Amounts of 5 to 8 per cent of the weight of the casein are sufficient to render it insoluble. Hexamine, when of good quality, has the appearance of granulated sugar; it is readily soluble in water, and is free from the fishy odor of other amines. It releases somewhat over its own weight of formaldehyde when treated with dilute acids. Aldehyde ammonia is of a similar character, being the acetaldehyde condensation product with ammonia. In the trade these aldehyde materials which cause casein and similar substances to become insoluble are referred to as "insolubilizers."

Proportion of Lime to Casein.

In the preparation of cold water paint more errors are probably made in proportioning the lime to the casein than are made in any other way. The solubility of the casein, when the paint is ready for application, is dependent on the alkalinity of the lime water, and this in turn is determined by the percentage of active CaO in the lime. Since commercial limes vary in this respect, due to variations in methods of manufacture, and to conversion to inactive $CaCO_3$ by exposure to air, it is of the utmost importance to determine accurately the actual CaO content by the sucrose [1] method or by titrating the lime with 0.1 *N* HCl, using phenolphthalein as an indicator.

Investigations of M. D. Swartz, using sulfuric casein and high calcium lime, have proved that for the best casein solubility the correct proportion of lime is 87½ pounds of CaO to 100 pounds of casein. In other words,

if 12 pounds of casein are used as the binder in each 100 pounds of dry paint, and if the hydrated lime contains 70 per cent of CaO, then 15 pounds of hydrated lime should be used. If the hydrated lime contains 50 per cent of CaO, then 21 pounds of lime should be used. Either more or less lime will render the product decidedly inferior.

This is exceedingly interesting and important and is perhaps the most vital point in the manufacture of casein water paints. In practical use it is quite essential that all of the casein present should go into solution so that the maximum binding of the pigment in the dried paint film will result. If insufficient lime is used, the casein will not dissolve readily and a chalky paint film will be obtained. If a large excess of lime is employed, the casein will dissolve to become very quickly reprecipitated and the binding of the pigment will be poor.

Testing Casein Paints.

Physical tests of casein paints are usually of greater value to both the manufacturer and the purchaser than are chemical analyses. It is probably for this reason that so little attention has been given by investigators to designing accurate methods for the analysis of cold water paints, although it is believed that this should be made the subject of an investigation by analytical chemists.

When determinations are made of the pigments in a casein paint the first step is generally to ash a weighed sample, and then to apply the regular methods of analysis designed for pigments in oil paints.[2, 3] Aside from determining the total CaO, it is necessary to determine the CO_2 content so as to estimate the amounts of CaO which are combined as $CaCO_3$ (whiting) and as calcium caseinate, together with any free hydrated lime. The CO_2 may be calculated as $CaCO_3$ and the CaO which is not in the form of whiting determined by subtracting the CaO equivalent to the $CaCO_3$ from the total CaO. This CaO may be calculated as hydrated lime, $Ca(OH)_2$, and reported as such.

With the ordinary run of cold water paints where silicates are absent, it is possible to determine the uncombined CaO by digesting a sample in strong, hot cane sugar solution, making up to a definite volume, filtering, and finally titrating with 0.1 *N* acid, using methyl orange as an indicator. Under these conditions $Ca(OH)_2$ readily forms the soluble calcium saccharate, which is easily hydrolysed by mineral acids, and CO_2 causes no interference in securing a sharp end point in the titration.

The actual amount of commercial casein present in casein paint is best obtained by determining the nitrogen content by the Kjeldahl method and then multiplying this nitrogen value by 7.70. The determination may be made on a 5-gram sample.

Physical Tests of Casein Paint.

The physical tests of cold water paint should include the following:

Specific gravity.
Volume.
Tinting strength.
Absorption percentage.
Settling property.
Degree of fineness.
Opacity.
Durability.
Washability.

Space does not permit of describing these tests in much detail. Specific gravity is determined by means of a specific gravity bottle, using benzine as the displacing medium. The specific gravity may also be expressed as weight per cubic foot. If the paint is in paste form it is necessary to determine the gravity by weighing a cubic inch of the paste which must be firmly pressed into a container holding the desired volume.

Volume is the term used to express the weight of a given bulk of loose material, and is always associated with dry powders. It is also referred to as the "apparent specific gravity." A convenient method of determining volume is by the Scott Volumeter, which employs a cubic inch measure. Results may be expressed as pounds per cubic foot, pounds per gallon, or in any other desired manner.

Tinting strength is determined either by mixing the white with a definite small percentage of lampblack, or, in the case of colored pigments, mixing the latter with a definite quantity of zinc white. This determination is made in comparison with type samples. Water is used as the vehicle and the paint is rubbed out into a paste with a spatula, on sized paper, glass or wood.

Absorption is determined by weighing out 20 grams of the sample and gradually adding water from a burette, until a stiff paste is formed. It is expressed as a percentage based on the weight of the sample used.

Settling property may be determined by taking a 100-gram sample, titrating with water to the consistency necessary for brush flow, and then observing the degree of settling in 24 hours.

Degree of commercial fineness may be obtained by using a Rotap sifting device, with sieves of 50, 100, 150, and 200 meshes per inch. The sifting may be aided by the use of steel balls $\frac{7}{16}$ of an inch in diameter. The actual fineness can be determined only by means of the microscope.

Opacity is determined by comparison with type samples. For all practical purposes the best comparison of whites may be made by brush-

ing out the paint on concrete cement, as on a clean, smooth, concrete floor, and noting the results after the paint has dried for 12 hours. Many more gradations may be observed than on glass or metal. Brush marks are best determined by brushing out the paint on a galvanized metal plate. Hiding power is determined by painting over a black surface; and covering power by brushing out a given quantity of paint and noting the area covered.

The durability of a paint may be determined by coating boards and slabs of slate with the paint and exposing the samples to the weather for a certain length of time. In this way the action of light, air and water may be observed and in some instances it is necessary to test the effects of deleterious gases, salt water, etc. In making the test with boards or slabs the painted surface should be placed in a nearly vertical position.

When a casein paint is applied to parchment paper and permitted to dry, it should not peel off when the paper is folded. If it flakes readily there is an insufficiency of binding agent. When brushed on a sheet of writing or printing paper, and permitted to dry, shrinkage or curling up indicates too much binder.

Washability may be determined by washing with a wet sponge a painted surface which has been permitted to dry for two days. If the paint rubs off it is not considered washable. The test may be extended to the use of water with mild alkaline cleansers. When the paint is applied to parchment paper, the dry film should withstand the action of a slowly moving stream of water for 6 hours, in order to be classed as waterproof.

U. S. Navy Specifications.

Those portions of the Navy Department Specifications, issued April 1, 1919, which relate to the quality of cold water paints are as follows:

Cold water paint shall consist of a uniform mixture of whiting of good grade, free lime, and casein, with zinc sulfate as preservative. It shall contain between 5 and 10 per cent of free lime (figured as calcium hydrate—$Ca(OH)_2$); 10 to 12 per cent of commercial casein (factor $N \times 8.1$); and not more than 1½ per cent of siliceous matter.

Cold water paint shall be capable of readily remixing at the end of 15 hours after having been mixed in proper proportion with cold water, and shall not possess an offensive odor.

The material shall be of white color and shall not chip, scale, powder, or rub off when washed with cold water, and in these respects shall be equal to the standard sample, portions of which may be obtained upon application to the Construction Officer at the navy yard at which delivery is to be made.

The U. S. Army specifications are nearly the same as those of the Navy but include the following requirement:

Must mix readily with hot water, and shall not possess an offensive odor after standing 72 hours at room temperature. When applied to a smooth surface shall flow evenly and cover solidly in one coat.

Physical Characteristics of Casein Paints.

Casein paints, whether applied to paper, wood, or metal, dry without gloss, which makes their use highly desirable for many purposes. Their ease of application makes them peculiarly adapted to certain classes of work, such as stucco, where an oil paint cannot be used advantageously. The time, labor, and cost, inclusive of material, are much lower than when oil paint is used. Casein paints are easy to work, dry quickly, and require no special preparation of the surface to be painted, except that it is sometimes necessary to remove a previous worn-out coating of the same kind on flat surfaces. Casein paints do not close the pores of brick work, but permit the bricks to "breathe." When properly prepared they are washable and will not rub, peel or flake off, and they are also weatherproof and fire retarding.

The following are the principal properties which determine the marketability of a water paint for general use:

1. Ease of preparation: A customer wishes to take the material, mix it with cold water, stir it as little as possible, and apply it immediately. He does not want the trouble of getting hot water, does not want to let it stand any length of time, nor does he want to go to any extra exertion. The paint must be quickly and easily prepared, which means that the materials must be ground extremely fine and intimately incorporated with the alkaline dissolving medium.

2. Ease of application: The paint must be so prepared that the layman can apply it easily. It must not run on the wall, must not streak, and must not pull under the brush. To insure that it will not run the casein binder must be reinforced with some slow moving plastic material, such as clay, asbestine, or a small amount of starch, which keeps it from being too fluid. If streaks are to be avoided it means that when the paint is brushed out the successive brush marks must flow together. This may be brought about by a proper blending of the plastic materials, such as starch to clay, to secure a nice smooth flow. This also insures ease of brushing and the absence of pulling under the brush, which is shown when lime and whiting only are used with casein.

The covering power of a water paint will depend not only upon the composition of the paint, but also upon the nature of the surface painted. The amount of water added to the paint will naturally be that amount which will give the best brush flow. The ordinary distemper or wall

finish, designed for application to plastered walls, wood, wood meal paper, wall board, etc., will cover 30 to 80 square feet to the pound of dry paint. A mill white, especially adapted for finishing the interiors of factories, mills and warehouses, where a clean, sanitary and improved light reflecting effect is desired, will cover 40 to 60 square feet. An exterior cold water paint, mixed in the proportion of 10 pounds of dry paint to one gallon of water, will cover from 25 to 80 square feet to the pound of dry paint, depending upon the surface to be covered.

Commercial Outside White.

The following is a practical working formula for outside white:

Whiting	55	per cent	for covering.
Clay	15	" "	for easy brushing.
Dextrine	2	" "	to prevent settling.
Casein	12	" "	for binder.
Lime	15	" "	for solvent.
Trisodium phosphate	1	" "	as added solvent.
Preservative	¾	" "	

In this formula the lime is figured as hydrated lime containing 70 per cent of active CaO.

Zinc sulfate has a good preservative action but tends to make the paint brittle and scaly. Bichloride of mercury is probably the best preservative. Two pounds of this may be added to 2000 pounds of whiting and ¾ pound of the mixture used in each 100 pounds of finished paint.

Casein Zinc Whites.

Some of the older manuals on paints give formulas for making casein flexible whites for application to paper, cloth, leather, etc. These formulas generally include water, ammonia, and glycerine as the solvents for the casein, and zinc oxide as the pigment. The addition of formaldehyde and an amount of ammonia sufficient to react with it chemically improves the drying qualities of the paint and makes it substantially moisture proof. These paints are often improved by the addition of more pigment such as lithopone. Sodium fluoride may be added to the solvent in small amounts, and when so used it is of considerable assistance with the higher viscosity caseins in producing a paint of good flowing properties. It may also be used to advantage in casein paints which are made up essentially of casein, lime, whiting, and clay, or other filler; and when so used should form about one-half per cent or more of the dry paint.

On the basis of the formulas for casein flexible whites, paints may be compounded which are suitable for painting stone and wood. The substitution of titanox for the zinc white does not produce a good paint,

but titanox may be successfully added to zinc white to secure acid proof and durable properties in a casein paint.

Oleo-Casein Paints.

An oleo-casein paint is essentially a water paint which combines oil with water as the chief constituents of the vehicle. Opaque gloss paints for application to wood, metal, stone, and stucco, may be made up on the same principle as the casein flexible whites, using linseed oil in the proportion of 25 to 50 per cent of the water vehicle. Raw, boiled, or burnt oil may be used, according to the character of the surface to be painted, the nature of the pigment used, and the time which may be allowed for drying in the particular work in hand. Boiled linseed oil is perhaps the most practicable for most purposes.

Oleo-casein paints spread well and are durable and very satisfactory in opacity and waterproofness. The paint in its liquid form has a tendency to settle, but the addition of a very small amount of sulfonated castor oil, or other sulfonated oil, such as soya bean oil, has been found of considerable value in holding the paint in suspension. This may also be effected by a proper combination of plasticizing agents, such as clay, asbestine, starch, etc. These are all matters which may be left to the chemist and paint maker to work out, and no published data or practical working formulas in this particular field can be cited. The marketing of oleo-casein paints in paste form for ready mixing with water, and their application by brush or spray are matters which still require considerable research for their development.

Latex-Casein Paints.

Latex-casein paints have been tried on paper, cloth, and leather with a considerable degree of success. They have a field of application in painting concrete and brick work, and particularly for road marking. The proper proportioning of rubber latex with caseins results in an increase in the life of the binding agent, and maintains the flexibility of the paint over a longer period of time than when it is absent. The presence of ammonia in the latex of commerce does not make the latex incompatible with the solvent medium for the casein but substantially assists it. In a formula for a casein flexible white it was found that the latex not only increased the flexibility but made the paint substantially waterproof. Road marking paint may be made by using a combination of casein, lime, drying oil, pigment and latex, the latter being first combined with the casein.

Specialty Paints.

It is probable that the most profitable field in casein paints is in the specialty line. Many of the formulas which are listed in books on paints

are of so little value that they are not worthy of repetition; patent literature also reveals little of any considerable value. However, some very good specialty paints have appeared on the market, including gloss enamel, sodium silicate, fireproof, moulders', stenciling, street marking, and stucco water paints.

Fireproof paints sometimes have phosphates as the basic fireproofing material, and are of considerable value for fireproofing stage curtains and scenery.

Steel moulders' paint is prepared by intimately mixing a little pulverized clay and rye flour with a small amount of casein and sufficient borax to make the casein soluble. The compound is boiled with water, and brushed or sprayed on the moulds, either before or after they are dried. The object is to produce castings with clean surfaces.

Sanitary calcimines containing waste lime borax sludge, whiting, zinc white, lithopone, and zinc formate combined with a plasticizer and casein have been proposed. A formula of this description employing the sludge obtained in refining borax is described by Carlton Ellis.

The demand for efficient street marking paint has come about through the institution of improved methods of guiding automobile traffic in town and country.

While there have been many objections to the use of cold water paints for anything but walls and ceilings, it is not unlikely that further investigations may prove that the most durable of floor paints may be designed with casein and lime with a certain amount of linseed oil or other oil as a base. The present oil-pigment, and silicate-magnesium oxychloride paints, have not proven serviceable for factory concrete cement flooring. A durable casein floor paint soluble in water, would not only prove simple to apply but would reduce the cost of floor painting by more than half. It may prove practicable to market such paints in paste or pulp form.

Manufacturing Casein Paints.

The methods of manufacturing casein paint in dry form are naturally quite different from those used in preparing oil paint. The equipment generally consists of a dry mixer, high speed pulverizer or stone mill, and a combined sifter and mixer. In the sifting operation the soft lumps are broken up and the whole mass is reduced to a uniform powder by brushes which rub against fine wire gauze. The fine portion drops into the mixer which has a set of revolving curved blades. This type of apparatus is the same as that used for blending batches of pigment for standard tints and for dry mixing preparatory to grinding for oil paint manufacture. The overtails from the sifter are sent back to the pulverizer.

Certain cold water paints are prepared in wet form, dried, and the

broken cake repulverized. While this method produces a high-class paint, it is limited to those which can be easily redissolved. Paints which are prepared in pulp form for packaging can be made in edge runner mills. In preparing dry casein paints it is best to grind and sift the casein first; the finer it is ground the better the product because of its quicker solubility. The proper amounts of lime and casein are then thoroughly mixed and placed in a large dry mixer, together with the "white covering" and plasticizing materials. The plasticizers, such as starch, are used in small amounts to "full" the paint. The materials are all thoroughly mixed and then into this mixture may be introduced phenol, or other preservative. After the mixing, all of the ingredients are passed through a stone mill or pulverizer and again ground for better incorporation. The material is then sifted, returning overtails to the pulverizer, until all is of the right degree of fineness, after which it is re-mixed and packaged.

Packaging Casein Paints.

Casein paints, as well as ordinary cold water paints, are put up in 5-pound, paper-lined packages, there being twenty to a case. In larger quantities they are packed in boxes, kegs or drums, containing 25, 50, or 100 pounds, or in barrels holding 350 pounds. Over 90 per cent of the casein paint sold is white, the remainder being made up of various tints, principally reds, grays, browns and creams.

In Europe cold water paints are marketed as semi-pastes, liquids or powders. While a considerable amount of the paint sold in Europe is in the semi-paste form, practically all of that marketed in the United States is handled as a powder. The semi-paste has a big advantage over the dry powder in that it is possible to secure better manufacturing control of the materials, better incorporation and better casein solubility by mixing and grinding wet at the factory. As a result the painter who stirs the paint in water is well satisfied, as the time required for its preparation is very small.

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Chapter 5

Casein in Paper Making

By E. Sutermeister

The use of casein in the paper industry is confined very largely to the preparation of coated or enameled papers, though there are a few other lines in which it is, or has been, used to a slight extent.

Nature of Coated Paper

Coated paper, which is also termed glazed, enameled or art paper, was developed within a comparatively recent period to satisfy the demands of printers for a paper upon which fine half-tones could be reproduced with good results. Such paper is prepared by mixing mineral matters with a solution of an adhesive and applying this mixture in a thin, even layer to the surface of an ordinary sheet of paper. The function of the adhesive is to bind the mineral matter so firmly to the paper that it will not be removed or "picked" off during the printing operation, while the mineral matters form a surface which is receptive to ink. Such a coating covers the individual fibers on the surface of the paper and also fills any hollows between them, so that the paper, after calendering, has a smooth, even, and continuous surface, which permits the finest dots of the half-tone plates to reproduce perfectly. Coated papers are used for lithographic work, for magazine and other printing, and more especially for high-class advertising where the perfection required of the illustrations necessitates the use of half-tone plates with a large number of lines per inch.

Adhesives Used

There are at present, three chief adhesives used in the coating of paper, starch, glue, and casein, but the amount of casein used far outweighs that of the other two combined. There has been a strong prejudice against starch because many coaters have attempted to use it without sufficient knowledge of its properties or the effects of modifying it by various chemical treatments. This resulted in the preparation of much paper which was weak coated and would not print without picking, and as a consequence starch in coating has been regarded as an evidence of

inferiority. This is very unfortunate, for when properly treated and applied starch will give as strong and permanent a coating as either glue or casein and moreover it is not subject to putrefactive decomposition with resulting bad odors. Coated papers in which starch is the adhesive seem to absorb the printing ink a little more than those with glue or casein coating, and while this necessitates the use of a little more ink it is not considered a serious defect.

Glue was practically the only adhesive used for some time after the development of coated paper. At that time it was found to vary considerably, even in shipments which were supposed to be the same, and it frequently resulted in weak coated paper and defective printing. Another trouble with glue was the objectionable odor which it often imparted to the finished paper. This was sometimes due to the spoiling of the glue solution in the coating plant and sometimes to the inferior quality of the materials used by the glue maker, but in either case the result was an unsatisfactory paper. Better controlled methods in the glue industry have gone far toward curing these troubles and a strong effort is being made to reestablish glue in the esteem of the paper coater. Whether this can be accomplished to any extent will depend considerably upon the relative cost of glue and casein.

Casein as an adhesive has one inherent advantage over either glue or starch—the ease with which it is rendered waterproof. Papers in which it is used can easily be waterproofed to such an extent that when moistened and placed in contact they will not adhere, nor will the surface be injured. If need be, the coating can be made so resistant to water that the paper can be washed with a sponge without injury. This same effect can be produced in glue coated papers, but only with difficulty and at considerable expense. Where starch is used there is no method known for making the coating waterproof. Apart from this advantage the chief reasons for preferring casein to glue are those of cost and the unwillingness of coaters to change their methods of working.

Attempts have been made at various times to prepare solutions containing both casein and starch, or casein and glue. Such solutions do not appear to be homogeneous, and, especially with starch, there is a decided tendency toward a curdling, or separation, of one or the other of the materials. In such a case the adhesive strength of the mixture is not equal to the sum of the strengths of the two components. In some instances it was even found that the starch did not add anything to the strength of the casein but acted merely as so much inert material. For this reason mixtures of the two are seldom used, though rarely a paper is found in the coating of which both casein and starch are present.

Early Attempts to Use Casein

Because of the destruction of records by fire it has proved impossible to establish the year in which casein was first used commercially in this country in coating paper, but it was probably between 1890 and 1900. Before that it is claimed that curd, or pot cheese, had been used on a small scale in several plants with excellent success so far as color, odor and adhesive strength were concerned, but that its use had to be abandoned because of the impossibility of preventing the curd from spoiling, especially in hot weather.

After the trials of green curd the next attempts were made with curd which had been dried and ground to the form in which casein is now found on the market. It was customary for the casein manufacturers or dealers to mix this casein with the alkali necessary for its solution before delivering it to the consumer, and such large quantities of borax and soda ash were used for this purpose that it almost seemed that the amounts were governed by the fact that the alkalies were cheaper than the casein. This practice left the paper coater no choice in the selection of his solvents and obliged him to work with very alkaline solutions. The high alkalinity of the dry casein also appeared to cause deterioration if it were stored for any length of time and this introduced another source of trouble. Little real progress was made in using casein until this custom was abandoned and the material delivered unmixed with alkali.

When casein was first used for coating paper it was being made as a by-product in many small creameries, and because it was a by-product little care was given to its manufacture. There was also no uniformity in the methods employed, some allowing the milk to sour itself, some adding acid and some using the rennet process. Sometimes the kinds of casein were kept separate and sometimes they were mixed, so that it was not only general to find the lots working differently, but it was not at all uncommon to find several barrels out of a carload which could not be used at all and had to be returned to the shipper. Under such conditions it was very difficult to locate the source of any trouble and the confusion was increased by the fact that the users had little knowledge of the way to handle casein and were more or less prejudiced against it. Doubtless many lots of casein were rejected which could be used to-day without difficulty, but in the early days they caused endless trouble and confusion.

Conditions are now greatly improved, both because the preparation of the casein is better standardized and looked after and because the paper coater knows much more about handling it to the best advantage. It is seldom that a lot of casein now has to be rejected because of poor quality, or even that unsatisfactory samples are received.

Coating Operations

In the preparation of coated papers five chief operations are involved, the dissolving of the casein or other adhesive, the mixing of the solution with the mineral matters to be used in the coating, the spreading of the completed mixture on the surface of the paper, the drying of the moist paper, and the smoothing or polishing of the surface by calenders or other means. The first three of these are influenced to a greater or less extent by the kind and quality of the casein used and knowing the degree and nature of these influences is a very great help to the paper coater in his attempts to produce a uniform and high-grade paper. The drying and calendering operations are common to all coated papers, whether casein, glue or starch is used, and as they are practically uninfluenced by the kind or quality of the adhesive they need not be discussed here.

Preparing Casein Solutions

The first step in preparing a casein solution is to mix the dry casein with water for a sufficient time to moisten each particle thoroughly before adding the alkalies. The time required depends on the temperature of the water as well as upon the fineness of grinding of the casein. Increasing the temperature of the water decreases the time necessary for its absorption by the casein, but the temperature must not be carried too high as this may cause the casein to soften and cohere in dense, sticky masses which are not easily penetrated by the alkalies and are, therefore, very difficult to dissolve. This takes place if the casein is added to water at 85° C. (185° F.) or over, but if the casein is mixed with cold water and the temperature then raised to the same degree, no such formation of masses occurs. It is probable that caseins differ in their tendency to cohere in this way, but no definite information regarding this property is available.

The fineness of the casein is also of considerable importance. If it is too coarse it requires an undesirably long soaking to permit the water to penetrate the coarsest pieces, while if it is too fine it is likely to lump up when it is mixed with water. Such lumps contain dry casein inclosed within a coating of wet particles. The alkali acts on only the outer portion and when the coating mixture containing such lumps is finally strained they break open and permit particles of undissolved casein to be distributed in the finished mixture. Such a mixture causes transparent spots in the paper when the particles of casein are crushed during calendering. It is only when nearly all the casein is finely divided that this trouble develops and a moderate amount of fine material, mixed with the coarse particles, does no harm. Screening tests on a South American

casein which worked very satisfactorily, and on a domestic casein which was considered to be too finely ground gave the following results:

	South American	Domestic
	Per cent	Per cent
Retained on 20 mesh	0.5	1.2
Passed by 20, retained on 40 mesh	32.5	16.2
Passed by 40, retained on 60 mesh	38.3	18.8
Passed by 60 mesh	28.7	63.8

Addition of Alkalies

After the casein is thoroughly soaked the next step is to add the alkalies; this is best done by dissolving them in a small amount of water and pouring the solution into the cooking vessel containing the casein. Among the alkalies which have been proposed are sodium sulfide, silicate, aluminate, sulfite, phosphates and carbonate, as well as borax, caustic soda and ammonia. Potassium salts would doubtless work equally well but they are never used on account of the expense. Of the alkalies mentioned the first four are seldom used on a commercial scale and the bulk of the work falls on the other five. The selection of a solvent depends to some extent on the personal preference of the coating room foreman but to a still greater extent on the type of paper being made. Borax cannot be used to any very great extent when satin white (a pigment made by treating slaked lime with aluminium sulfate) is present in the coating because it causes a considerable thickening of the coating mixture. It is desirable to use as much of it as possible, however, in order to take advantage of its preservative properties. Ammonia is a vigorous and rapid solvent and it has the advantage of being driven off during the drying of the paper, thus rendering the coating distinctly waterproof, as well as giving it a neutral or slightly acid reaction toward litmus. This makes it particularly valuable for certain papers which must be free from soluble alkali. Caustic soda is one of the cheapest alkalies to use because of its great solvent power, but if it is used in excess it causes the solution to darken, sets up decomposition of the casein and acts destructively on the brushes of the coating machines. For these reasons it is generally avoided in the preparation of the solutions but it deserves more attention than it gets and is safe to use wherever good technical control is maintained.

From the success attending the use of slaked lime as a solvent in making casein glues it would appear that it might have possibilities in paper coating work. It has the disadvantage of reducing the time during which the coating mixture retains its good working properties, and from the few experiments which have been made it seems to increase

very greatly the foaming properties of the coating. It is believed that this solvent is well worth studying in the hope of overcoming these handicaps.

From practical trials with a large number of caseins the pounds of solvent necessary for just complete solution of 100 pounds of casein has been determined. This is given in the following table, together with the chemical equivalent of the caustic soda necessary on the assumption that the solvent power depends only on the amount of base present.

Alkali	Pounds Solvent	Chemical Equivalent
NaOH	3.95	3.95
NH_4OH	3.15	3.45
$Na_2B_4O_7.10\ H_2O$	14.7	18.9
$Na_3PO_4.12\ H_2O$	12.3	12.5
$Na_2SO_3.7\ H_2O$	14.0	12.5
Na_2CO_3	5.14	5.23

It is seldom that any one of these solvents is used alone, as it is quite general to use a mixture of two or more which will give the desired properties to the coating mixture. These combinations of solvents have been made up from the results of practical trials and it has proved impossible to predict the effect of any given mixture from scientific considerations.

In preparing solutions for use in paper coating the chief consideration is to obtain a complete solution and any amount of solvent which will give such a solution will develop the full adhesive strength of the casein. It has also been shown that, so long as the solution is complete, the temperature of cooking is without influence on the strength of the casein, unless the heating is so prolonged as to cause its decomposition. It was found, for instance, that a casein dissolved at 20° C. (70° F.) was neither stronger nor weaker than the same casein dissolved at a temperature of 86° C. (195° F.) for 15 minutes. While such high temperatures are safe in laboratory work they should never be permitted where large quantities of solution are being prepared, because it requires so much time to cool a large body of solution that decomposition may have taken place to a considerable extent before a safe temperature was reached.

The amount of solvent used in preparing solutions for paper coating is generally considerably in excess of that required for a neutral or complete solution. With most of the solvents this excess of alkali does no harm, and it has been found necessary when much satin white is used. When ammonia is employed in considerable excess it darkens the solution to a marked degree and often tends to thicken it. Caustic soda in

excess is very apt to cause decomposition of the casein into substances darker in color and of little adhesive strength. It is difficult to see how any workable solution could have been obtained by a Dutch formula of 1911, in which 15 parts of casein and 15 of caustic soda were dissolved in 70 parts of water. Such a solution must have had remarkably poor keeping qualities and been extremely destructive to the brushes.

The preparation of casein solutions is usually carried out in open tanks fitted with agitators. They are preferably of such shape that the solution may be drawn off rapidly and completely. The agitators should be of a type to give good mixing without splashing, for this will spatter the casein onto the sides of the tank where it will gradually collect and decompose, making it difficult to keep the apparatus clean and sweet. Formerly the heating was done by blowing steam directly into the contents of the cooker, but the more modern method is to cook in steam jacketed tanks. This permits a better control of the density of the solution, since no water is added by the condensation of the steam, and it also makes it possible to cool the solution by passing cold water through the jacket. This has been found desirable because it gives lighter colored solutions, and prevents decomposition of the casein when large amounts of alkali are used. Practical experience has shown that satisfactory solutions may be obtained by agitating at a temperature of 54° to 60° C. (130° to 140° F.) for an hour. As soon as it is certain that the casein is all dissolved the steam should be shut off and the solution cooled to 40° C. (100° F.) or below. This is particularly necessary when the casein is to be mixed with satin white, for if the solution is too hot the mixture thickens so greatly that it cannot be applied to the paper.

Where ammonia is used in conjunction with other solvents some authorities claim that it should not be added until the full temperature of cooking has been reached and the steam shut off. This reduces the loss of ammonia through volatilization and permits it to act on the casein when the latter is thoroughly softened. In other plants this rule is not observed and the ammonia is added with the other solvents when steaming is started.

Typical Mill Procedures

In the preparation of casein solutions in the coating plant it is not always practicable to observe all the general rules just set down and in fact they would not all be subscribed to by every paper coater. It may therefore be of interest to describe three or four actual mill procedures. For the sake of convenience all the formulæ in the following table have been calculated to a basis of 100 pounds of casein.

In the plant using formula No. 1 Clark [1] states that the casein is soaked in water one hour and the borax and trisodium phosphate, dis-

Formula No.	1	2	3	4
Casein, lbs.	100	100	100	100
Borax, lbs.	5	4.5	3.2	5.5
Trisodium phosphate, lbs.	6.4	29.6	20.8	7.4
Soda ash, lbs.	...	5.5	...	...
Ammonia, 26°, gals.	2	0.4	0.4	0.4

solved in water, are then added to it. The mixture is heated to 150° F. by passing steam through the jacket; steam is then shut off and the ammonia added. Cold water is then passed through the jacket until the temperature of the solution drops to 80-90° F. Agitation is continuous during cooking and cooling in order to avoid the formation of "skins" on the surface of the solution.

The plant using formula No. 2 adds the casein to the proper amount of water and as soon as it is thoroughly agitated and all casein is wet the dry solvents are added. Steam at 90 pounds pressure is then blown directly into the charge until the temperature reaches 130° F., when it is shut off. During the heating period the ammonia is added at the convenience of the workman and as soon as solution is complete the charge is cooled to 95-100° F. The last of the cooling is done without agitation to avoid whipping air into the thickened solution.

Formulæ 2 and 3 have been used with good results when a considerable amount of satin white is present in the coating, while No. 4 works well with clay alone but is not satisfactory with satin white.

All casein contains more or less dirt and insoluble matter and in some cases it is desirable to clarify the solution before use. This can be done very satisfactorily by passing the hot solution through a centrifugal separator in which the bowl is so constructed as to retain the dirt. This treatment makes it possible to use caseins which would otherwise be unfit for use in high grade papers.

Preparing the Coating Mixture.

After the casein solution is prepared the next step in the process is its incorporation with the mineral substances and other materials to produce the finished coating mixture.

The general method of preparing coating mixtures is to work up the mineral matters and water in an agitator which will thoroughly incorporate them, and then add the casein solution. The tinting materials and the foam reducers are added just before the mixture goes to the coating machine. For reasons of economy it is desired to use as little casein as possible and still hold the coating on the paper strongly enough to resist the pull of the printing ink. This is also desirable because

the more casein used the less gloss the paper will take on calendering, and the less readily will it absorb printing ink.

Mineral Matters Used

The mineral matters for the great bulk of coated paper consist of white pigments such as china clay, blanc fixe ($BaSO_4$), precipitated chalk ($CaCO_3$), satin white, talc, etc. With these are mixed coloring matters of various kinds to give the tints or colors desired in the finished paper, and waxy or oily substances to improve the finish of the paper or reduce the foaming tendencies of the coating mixtures.

The minerals used vary with the kind of paper which is to be made; blanc fixe and precipitated chalk give dull or semi-dull finishes, clay a more glossy surface and satin white a still higher shine. Apart from these differences they have also a distinct influence on the amount of casein necessary. This appears to depend partly on the fineness of the particles and partly on their chemical nature. Because of these variations it is difficult to give accurate comparisons of the amounts of casein required by different minerals but from laboratory tests 100 parts of dry mineral matter appear to require about the following amounts of casein:

Blanc fixe	6 to 7 parts
Precipitated chalk	8 to 10 "
Clay	7 to 11 "
Satin white	12 to 16 "

For some unknown reason the quantities necessary in actual practice are considerably greater than these, but the relative amounts remain about the same.

The effect of degree of fineness has been proved by separating a clay into portions remaining in suspension in water for various lengths of time, and testing the fractions. It was found that the finest part required several times as much casein as the coarsest. With four different clays, in which the portion remaining in suspension over two hours varied from 41.8 to 86.2 per cent, the casein required varied from a little over four parts to eight parts, increasing with the increase of the fine fraction in the clay. It is probable that the difference between clays is due to physical rather than chemical causes, but whatever the reason, it is quite possible that it is sometimes the reason for the casein being unjustly blamed for poor strength.

With satin white the fineness of particles may be responsible for the greater amount of casein required, over that necessary for clay, but here another factor enters—*viz.*, the chemical reactivity of the material. The actual composition of satin white is not yet known but it is formed by the interaction of slaked lime and aluminium sulfate and is

of a strongly alkaline nature. It has been found necessary to hold the alkalinity of the satin white, as expressed by direct titration with acid, within certain limits if trouble is to be avoided. If conditions are not right the coating mixture will thicken to such an extent that it cannot be spread. All of the reasons for this change are not known but it will take place if the alkalinity of the satin white drops too low, if borax is used as a casein solvent, if a considerable excess of alkali is not used with the casein and if the solution is too hot when it is mixed with the satin white. Caseins differ considerably in the degree to which they thicken with satin white and it is quite probable that a careful study of the situation would enable a casein to be produced which would be much less likely to cause trouble than the present commercial products.

Coating the Paper.

There are a number of types of coating machines, some of which coat one side of the paper at a time while others coat both sides at one operation. In the latter the paper is drawn in a continuous web through a trough of the mixture, then passes between squeeze rolls to remove all but the desired amount, and is then subjected to the action of brushes working back and forth across both surfaces of the paper. The brushes distribute the coating evenly and the paper is then dried over blasts of heated air and in festoons in a drying gallery.

During these operations several troubles are apt to develop which are caused, in part at least, by the casein. If the latter is incompletely soluble the undissolved particles are apt to work up into the brushes until they collect in such quantities that the bristles can no longer hold them. They then drop off onto the paper and form lumps in the coating which are ruinous to the printing plates if they are not all removed during the sorting of the paper. If the fluidity of the coating mixture is not right it will not give good results. When it is too thin it may prove impossible to apply enough coating, while if it is too thick the surface of the paper may be full of brush marks. These are the marks caused by the bristles dragging through the coating; when its consistency is just right it is fluid enough so that it flows a little after it leaves the brushes and the marks disappear, but when it is too thick this flow is lacking and the tracks of the brushes show in the finished paper. The question of proper fluidity in the coating mixture comes down to the selection of the right solvents to go with the casein and minerals employed.

Foaming

A third trouble which is probably caused to a considerable extent by casein is that of foaming. If the coating mixture becomes very frothy

it is likely to cause innumerable small pits in the coated paper, which may, at times, be bad enough to spoil its printing qualities. All of the factors which cause the formation of foam are not known, but the fact that it varies with different caseins when all other conditions are constant throws considerable suspicion onto the casein. Even in laboratory tests it can be proved that caseins vary widely in foam producing power, but the reason for this variation has never received any great amount of study. In the coating industry the foam is kept down to a safe point by the use of some material which causes the bubbles to break as soon as they are formed, but even this is not always successful and the use of such materials sometimes introduces troubles which are as bad as froth. It would probably repay the makers of casein to study the causes of frothing and the means for its elimination from their product.

Defects in Casein.

Casein as placed on the market is subject to several defects, most of which can be eliminated if proper care is taken during its preparation. It is only fair to say that they are much less frequent than formerly, indicating better cooperation on the part of producer and consumer.

Odor in casein cannot be entirely avoided as it has a characteristic odor when prepared by commercial methods. That it is due to impurities is indicated by the fact that pure casein, as prepared by laboratory methods, is nearly odorless. Unless handled very carelessly in its manufacture, thus permitting decomposition to set in, the odor of casein is not bad enough to be objectionable to most persons, but if putrefaction begins, either during the making of the casein or during its use in the coating plant the odor of the finished paper may be quite objectionable.

If casein is dried at too high a temperature, or for too long a time it may become discolored and difficultly soluble. Red-brown or "burned" particles in casein used to be a frequent cause of complaint but of recent years they are seldom seen. It was generally the case that only part of the casein was thus discolored and the rest was of normal appearance. This is a bit difficult to account for, considering the uniform treatment given the curd before and during drying.

If casein is purchased in the form of coarse curd and ground at the point of use trouble may be experienced if the drying has not been carried far enough. Sometimes the larger lumps contain cores of moist material which is of a rubbery consistency and resists the disintegrating action of the grinder. This is a very unusual form of trouble.

It is claimed that an excessive amount of fat in casein will cause trouble in the coating by producing "eyes" or grease spots. There is no reliable information as to the amount of fat which is permissible in a satisfactory casein and it probably varies with the type of work being

done. Buttermilk casein contains more fat than that made from skim milk and one instance is known in which a lot containing 12 per cent of fat was used without trouble. This was in the early days when the quality of paper was not up to that made now, and there is some doubt about casein of that quality being used successfully at present.

A frequent cause for complaint used to be the presence of white flakes, which were sometimes visible in the dry casein, and which would not go into solution when it was dissolved. These were often attributed to albumen, precipitated with the casein because of too high a temperature when treating the milk with acid. It was finally proved that they consisted of the hyphæ of molds and that they were present because the drying of the casein was so long delayed that the moist curd had a chance to mold. Proper handling of the curd entirely eliminates this trouble and it is unusual to find white flakes in modern caseins. This defect in casein is the cause of two troubles in coated paper: the white flakes cause little spots in the paper which do not print well, and they sometimes work up into the brushes of the coating machines and collect until in sufficient quantity to fall off and make bad spots in the paper.

The foaming of coating mixtures has already been mentioned. This may or may not be due to defective casein, but it is strongly suspected that the impurities in the casein, or perhaps some of its degradation products, are largely responsible for its foaming. It is believed that this would be a fruitful field for research.

The deterioration of casein during storage is a fault which cannot be laid entirely to the casein, though some kinds keep better than others. If casein is stored in a warm place it is likely to become infested with worms and after a while such casein will be found to have lost much of its strength and to contain considerable amounts of insoluble matter. In some cases caseins have been known to lose half their adhesive strength and to give such thin, watery solutions that the coating mixtures made from them were not of a workable consistency. The storage of caseins will be discussed in a later chapter.

Preservatives.

When casein was first used in paper making much trouble was caused by the casein spoiling in the coating plant. This resulted in very bad odors around the plant and in the paper itself, the latter feature being bad enough to cause much complaint from its users. As the putrefactive spoiling of the casein also reduced its strength to a very marked extent numerous lots of weak coated paper were sent out with disastrous results.

For a time the cause of this trouble was not fully realized and attempts

were made to find a preservative or deodorizer which would not injure the quality of the paper. All sorts of disinfectants were tried and fair results obtained with camphor and some of the essential oils. The use of borax was also found to be very beneficial in preventing the spoiling of the solutions, and its use became very general. Gradually, however, the idea gained ground that more cleanliness in the coating plant and more care on the part of the casein maker would avoid this trouble, and in recent years there has been no cause for complaint regarding the putrefaction of casein in process. It is true that there is still a slight odor in paper coated with casein, but it is that due to the casein itself and is not caused in any way by spoilage. Occasionally an individual is found to whom this odor is objectionable but to the great majority of persons it is hardly noticeable.

Waterproofing Agents.

When the casein is dissolved by sodium salts and used in a clay coated paper the finished coating is not waterproof and may be removed easily by rubbing with a wet finger. With such papers moisture causes the sheets to stick together so firmly that a book or pamphlet may be entirely ruined. This fault can be overcome by rendering the casein insoluble after it has been applied to the paper, and it was found that there were various means of accomplishing this result. Treatment of the coated paper with a solution of such materials as ferric chloride, lead acetate, copper sulfate, chrome alum, zinc sulfate, etc., causes the formation of the caseinates of the metals in question, and as these are insoluble the paper becomes waterproof to such an extent that it can be washed with a sponge. None of these materials can be added directly to the coating mixture before applying it to the paper so their use involves an additional step in the manufacturing process, which is undesirable. Also many of the caseinates formed are colored, which prevents their use in white papers.

The most successful method of making the coating waterproof involves the use of formaldehyde and this is extensively employed in those grades of paper where the use of satin white is not general, as, for instance, in papers for covering boxes. It was found that if the formaldehyde were considerably diluted it could be added to the coating mixture without causing any change in its consistency, but that after this was applied to the paper and dried it gradually became waterproof. Too much formaldehyde or too strong a solution caused an immediate thickening of the coating mixture and apparently part of the casein was rendered unavailable as an adhesive, for it was found necessary to use more than the ordinary amount in order to make the coating strong enough. About a pint of commercial formaldehyde for 100 pounds of casein caused the

coating mixture to thicken only very slightly but after standing one day it could not be thinned down by reheating. In practice rather less than this was found to develop sufficient waterproof qualities after the paper had stood for a short time.

Attempts have been made to use other materials, especially furfural and hexamethylenetetramine, as waterproofing agents. Furfural has not proved valuable for this work but hexamethylenetetramine has been used quite extensively and with considerable success. It is customary to prepare it when needed by mixing about equal volumes of formaldehyde and 26° ammonia; toward the end the ammonia is added very cautiously until the mixture has the odor of neither constituent. This mixture can be used in much larger proportion than formaldehyde without causing the coating mixture to thicken, and when subjected to the heat of the drying line it is broken down into its two constituents. The formaldehyde thus produced causes the waterproofing of the coating. Waterproofing in this way is not so important as formerly because satin white is much more generally used and its presence in the coating tends to make it waterproof. This is probably due to the formation of insoluble calcium caseinate from the lime salts in the satin white. Doubtless the same result could be accomplished by the use of slaked lime as a solvent, if a method could be devised for employing it successfully. In some cases the use of ammonia as a solvent adds to the waterproof qualities of the coating, for the ammonia is driven off during the drying operation and the casein remains in an insoluble condition.

Other Uses of Casein

The addition of an alkaline solution of casein to the paper stock, followed by the addition of alum, causes the precipitation of the casein on the fibers. This treatment is carried out in the beater, which, with respect to this process, acts merely in the capacity of a mixing device. The precipitated casein aids in cementing the fibers together and in preventing the fuzz which sometimes sticks up from the surface of the paper. It does not assist in retaining clay or other filler, nor does it improve the resistance to penetration of the varnish which is sometimes applied in lithographic work. Papers with casein are firmer and have more snap and rattle than those not so treated, and it is probable that their strength is increased, though the evidence on this point is somewhat contradictory. There is a distinct tendency for casein in the beaters to cause foaming, and in some cases this has proved to be rather serious.

In the past considerable quantities of casein have been used in the beaters in the production of the so-called "onion-skin" grade of writing paper. There is no question about the good results which it gives in

this paper, but the benefit derived is not proportionate to the cost of the casein, and its use has been entirely abandoned in favor of cheaper materials. So far as known this is true of its use in the beater for other grades and it is safe to say that the amount consumed in such ways is extremely small.

Casein has been proposed as an aid in the rosin sizing process which imparts to paper the property of resisting the penetration of writing ink or other aqueous fluids. Here again contradictory results have been obtained. In one test where the rosin and casein were made into a size together, 10 per cent of casein of the weight of the rosin being used, the sizing was no better than when no casein was employed. If the casein is added to the beater to act as a dispersing agent and cause the precipitation of the rosin in colloidal dimensions it appears to improve the sizing very distinctly. One per cent on the weight of the materials in the beater increased the sizing of the sheet to 300 per cent of its value when no casein was present, while 0.5 per cent of casein increased it to 160 per cent of its original value. These results are based on tests made by floating pieces of paper on a bath of writing ink and noting the time required for the ink to show on the upper surface. As strongly sized paper can be made more cheaply by other means casein is seldom used for this purpose.

Some years ago attempts were made to use casein as a surface sizing agent for writing papers in place of glue. For this work the paper is run through a dilute solution of glue and then dried in any suitable way. When casein was used in alkaline solution, and properly diluted, it would give a fairly good snap and ink resistance at first, but as the web continued to run through the bath of casein solution the acidity of the paper, due to the presence of alum, gradually neutralized the alkali and the casein began to precipitate in small flakes which spotted and ruined the paper. Continual addition of alkali would doubtless have overcome this difficulty, but the writing paper mills never favored the process sufficiently to justify further trials.

According to a recent report from the Department of the Interior at Ottawa,[2] casein can replace fish glue in the making of strong and durable waterproof and fire-proof asbestos paper, board, etc., and also in the preparation of high pressure steam gaskets and the facing for brake linings. It is probably not very extensively used for such purposes, for it has been ascertained from three of the largest makers of asbestos products that they do not use it in any of their work.

Up to a short time ago casein was used to some extent for various purposes in the wall paper industry. It served as a finishing coat on certain heavy goods, colored and embossed in imitation of leather, and in the papers known as "varnish tiles" it was used as a size coat to sup-

port the varnish. For this latter purpose its increasing cost made it too expensive and it was replaced by a vegetable product. One manufacturer of wall paper states that casein has no real advantages in that industry because of its odor, and because its use in alkaline solution would have a detrimental effect on colors and on the ground wood in the body of the paper.

In the manufacture of playing cards casein is used to some extent for the same purposes and in the same way as in the coating of paper. It is also used in conjunction with shellac dissolved with alkali as a top varnish for playing cards, for the top coat of two-coat waterproof papers, and for sizing in hard sized sheets. It can also be used, with an after treatment with formaldehyde, for oil- and waterproofing art papers, cartridge cases, buckets and bags, etc. While it may be used in small quantities in the production of these, and other paper converting specialties, the amount so consumed is insignificant compared with that employed in the coating of paper, and a description of the latter industry is therefore fairly representative of the use of casein in paper making.

Kinds of Casein Used.

As explained in the previous chapter casein is prepared by treating the milk with rennet, with acid, or by allowing it to sour itself. Each of these processes imparts certain characteristics to the casein and with any single process the method of carrying it out and the severity of the treatment are also influential. It is not to be wondered at, therefore, that the product varies more or less and that this was particularly true in the early days when there was almost no uniformity in the methods of production.

Of the various kinds of casein made, that produced by treatment with rennet is not used in paper coating because it is not sufficiently soluble in alkaline solutions. This was a frequent source of trouble when casein was first introduced, for rennet casein was then often mixed with that made by the other processes and the insoluble part made it impossible to use the rest.

Natural soured, or self-soured casein is at present the choice of the majority of paper coaters. The great bulk of the Argentine casein is made in this way and it has a high reputation for good working properties and uniformity. It gives a thin solution with little foam and the coating mixtures made from it are of good fluidity and spread well.

Casein precipitated by adding acid to the skim-milk varies considerably in its properties and it was formerly thought that this was due to the kind of acid used. This opinion was probably based on the observation that muriatic casein, or that precipitated by hydrochloric acid, gave much thicker solutions and coating mixtures than that precipitated by

sulfuric acid, and that both were of higher viscosity than those made from self-soured casein. A careful series of tests was carried out in 1922 [3] on caseins especially prepared by precipitation with different acids, sulfuric, phosphoric, acetic, lactic, hydrochloric and mixtures of the last three with hydrochloric, and so far as viscosity of solutions or coating mixtures was concerned comparatively slight differences were observable among them. The mixtures made with all of them were so thick that they could not be tested with the available viscometer, while that made from a self-soured casein was thin and fluid at the same temperature and gave a low reading for viscosity.

The ash in all these caseins ranged from 5.50 to 6.57 per cent while that in the self-soured sample was only 2.98 per cent. Tests were therefore carried out to see if treatment of the commercial caseins with hydrochloric acid would reduce the ash and, at the same time, the viscosity of the coating mixtures made from the treated casein. It was demonstrated that the ash could be reduced to 2.0 per cent, or even less in some cases, and that this change caused the coating mixtures to become nearly as fluid as that made from self-soured casein. Sulfuric acid was not nearly as effective as hydrochloric in reducing the viscosity and the amount of acid used had a great influence on the results; the more acid employed the lower the viscosity. The acid treatment causes a loss in weight of 11 to 15 per cent of the original casein, but the strength of the treated casein is enough greater to slightly more than counteract the loss in weight.

An electrolytic method of precipitating casein was tried out some years ago by one manufacturer and it is said that a high grade product was obtained. Unfortunately the experimental plant was put out of commission by an accident and as it was quite expensive to install no further experiments were made.

Buttermilk casein has been used to some extent in paper coating and with very variable results. Some lots would be of light color, and good solubility and strength, while others were reddish brown and contained so much insoluble matter that they could not be used in the ordinary way. Even under those conditions the soluble part was of good strength and if the solution were clarified by centrifugal action it could be used with good results. It is quite certain that if it is properly made buttermilk casein can be used to good advantage in coating paper.

A study of the viscosities of casein solutions and coating mixtures was made by the writer in 1919 [4] and it was shown that the viscosity of a casein solution depended on the kind of casein and also on the kind and amount of solvent used. The same thing was true of coating mixtures containing clay and satin white but a casein giving a thin solution did not necessarily give a thin coating mixture, and it did not prove possible to grade a casein as to its value in coating paper by viscosity tests of

its solutions alone. The work already done has developed a valuable means of testing caseins for use in certain grades of paper but it should be carried very much further in order to develop its maximum usefulness. It is highly probable that an intensive study along this line would develop basic facts which would be extremely useful to the casein maker as well as the paper coater and that they would lead to a much more intelligent use of casein.

REFERENCES

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3. Sutermeister, E. "Casein and its Modification by Acid." Paper Industry, December, 1922.
4. Sutermeister, E. "Casein and Coating Mixtures." Paper, April 23, 1919.

Chapter 6

Casein Plastics

By George H. Brother

One of the latest industrial applications of casein to be discovered and developed is the preparation of plastic bodies or masses, but it bids fair to be one of the most important. An industry not yet thirty years old, which can show an annual (1925) production figure of around 3000 tons in one country alone, Germany, certainly has some basis for this claim.

The term "plastic" or "plastic mass" may seem rather ambiguous, especially in view of the fact that the material here considered is, at ordinary temperature, little if any more plastic, in the usual sense of the word, than wood. The material receives its name from its classification, which is technical rather than commercial. Through frequent use, however, during the period the material was being developed, the term "casein plastics" has come into general use.

There are a number of definitions as to what shall constitute a "plastic" body to be found in the literature of the subject, but the two which seem general enough and yet not ambiguous are: first, bodies which in process of manufacture soften sufficiently, usually, though not necessarily, under the influence of increased heat or pressure, so they can be pressed or poured to fill molds or forms; and, second, bodies rigid at ordinary temperatures and pressures, which under the influence of increased temperature or pressure become softened so as to permit bending or forming. The type of material considered in this chapter will subsequently be seen to conform to both of these definitions. It will also be found considered, along with nitro-cellulose (celluloid), other cellulose esters, albuminous bodies, synthetic resins derived from phenol or cresol condensation, rubber and rubber substitutes, shellac molding mixtures, tar, etc., in all modern periodicals or treatises on plastics.[1,2]

History of Development

The discovery of the adaptability of casein to the production of plastic material and its development into the industry of today is an interesting, though not unusual, chapter in the contributions of chemistry

to industry. The first record of any work done along this line is contained in a German patent, taken out in 1885 by Emery Edwin Childs of Brooklyn, New York, covering a process for the production of plastic masses from curd. Mr. Childs apparently did not realize the importance of his discovery, as there is no record of any development from it. According to Karl Wernicke [3] and Eugen Stich,[4] in 1897 there was a demand in school circles in Germany for a white "black-board." The publishing and lithographing company of Edler and Krische undertook the problem and tried, among other things, a casein coating on cardboard, but the results were not satisfactory. Krische secured Adolf Spitteler, a chemist, to work on the problem. In experimenting further with casein coating, Spitteler produced a tough, horn-like, insoluble mass, the importance of which was immediately apparent to both Krische and himself, so they protected themselves with patents, the most important being Ger, 127,942, the basic patent covering the fundamentals of the process. Two years of development work demonstrated the fact that a large capital and a staff of technical men would be required for success. Accordingly they induced the Vereinigten Gummiwarenfabriken of Harburg and Vienna to take up the proposition. In 1900 they sold the French rights to August Pellerin and Leon Orosdi and established the trade name "Galalith," from the Greek meaning "milk stone," for their product. In 1904 the French and German interests merged to form the Internationale Galalith Gesellschaft Hoff & Co. This company, the pioneer in the industry and the only manufacturer of the product until the lapse of the basic patent and the war made it possible for others to follow, is still one of the largest producers, if not the largest, in the world. So well have they established their product that their trade name, Galalith, is quite generally applied by the trade to any casein plastic, regardless of its source or individual trade name, and so it bids fair to become generally used, as have celluloid and linoleum in other industries. According to Wernicke,[3] in 1912 the Internationale Galalith Gesellschaft at Harburg employed 350 persons in a plant having a total floor space of 21,000 square meters and containing over 350 machines, largely electrically driven.

With the expiration of the basic patents, companies were formed in countries all over the world to manufacture these plastics. Numbers of these, not realizing the difficulty of the task ahead of them, were unsuccessful, but a substantial remainder have their product on the market. A partial list of these successful concerns, with the trade names of their products, is given on the following page.

All of these trade names represent casein plastic material, similar in composition and properties to the original Galalith and probably all produced by practically the same process of manufacture.

Company	Country	Trade Name
Deutsche Kunsthorn Gesellschaft.	Germany	Neolith
Erinoid, Ltd.	Great Britain	Erinoid
British Xylonite Co., Ltd.	Great Britain	Lactoid
Societa d'Esportazione.	Italy	Zoolite
Hollandsche Casolith-Werke.	Holland	Casolith
Akalit-Kunsthornwerke Akt. Gesellschaft.	Austria	Akalit
Compagnie générale d'electricité.	France	Lactolithe
Société industrielle de Celluloid.	France	Sicalithe
Aladdinite Company, Inc.	United States	Aladdinite
Erinoid Company of America.	" "	Erinoid
Karolith Corporation.	" "	Karolith
Kyloid Company.	" "	Kyloid

Mention was made above of the number of failures to produce casein plastics. In this country there is a large number of people who believe anything scientific, animate or inanimate, which comes from Europe, especially from Germany, to be superior to domestic talent or products. Those people have spent thousands of dollars in enterprises built on the formulas of Europeans claiming to know all about the very "secret" process for manufacturing casein plastics. These Europeans nearly always claim to have worked at, or to have some inside information regarding the processes in use at, the Internationale Galalith Gesellschaft plant. Without exception their enterprises have failed. The trouble has been a mistaken idea of the importance of formulas and processes, these being considered very secret and all that was necessary for successful operation. As a matter of fact, except for details which would naturally be worked out by the individual manufacturer for himself, these "very secret processes" are to be found in print. The essential for success is not so much the formula as the application of the formula; in other words, technique. And it does not detract in the least from the achievements of our brother scientists in Europe to state that the experience of this industry in this country has shown American chemists to be their equal when properly backed financially. Of the four companies at present producing in the United States, three developed their own technique through research conducted by Americans, and the remaining one secured its formulas from a reputable and established concern which sent skilled technicians to apply them.

Manufacture

There are two general methods which can be followed to produce this material, known as the wet and dry processes. They are both built around the fact that casein masses are rendered tough and insoluble by

the action of a so-called hardening agent; they differ fundamentally in the fact that the casein is put into solution in the wet process whereas in the dry process no such step is employed. Inasmuch as acid caseins are the more soluble in alkalies and the coagulum obtained from a dissolved casein solution is an acid casein, regardless of the nature of the original casein employed, acid caseins were used for the wet process product. It was found that rennet casein produced a better product by the dry process, so this class of casein is in general use in the industry at the present time, the dry process being the newer and improved process, as will be shown.

In order to produce a perfectly clear, transparent product, instead of a translucent one, it is necessary to give the casein a special treatment. Spitteler [5] patented a process consisting of treating an alkaline solution of casein with an excess of caustic. Upon standing, solid matter settles out, leaving a perfectly clear solution, from which transparent plastic material may be made. J. N. Reithoffer [6] patented a process consisting of treating skim-milk with soda ash, centrifuging and coagulating the casein from the clarified milk with acid. It is claimed transparent masses can be produced from this casein. Amandus Bartels has taken out patents which claim to attain this result by two methods,—first,[7] by mechanically rubbing freshly precipitated curd in order to destroy the "layer structure," and second,[8] by extracting powdered casein contained in a suitable machine (specified) with alcohol, benzene or a mixture of the two, to remove the last traces of fat. It is our opinion, although there is nothing in the literature to substantiate it, that all these methods have been superseded in the industry by one much simpler; a method taking into consideration the fact that casein is a colloid in nature and hence is susceptible to clarifying reactions, produced by the presence of a small quantity of certain salts.

The basic patent of Krische and Spitteler, mentioned above, specified the solution of casein in alkali, its coagulation with an acid and the subsequent treatment of the pressed mass with formaldehyde. Thus the industry was initiated with a wet process method and this is the course it followed in general during its early years. Wolter [9] describes such a process in detail, but his description indicates that he did not himself have first hand contact with the process, as some of the details are quite ambiguous. This fault seems to be rather general, especially in the patent literature.

The essentials of the wet process method of Krische and Spitteler are about as follows: An acid casein (muriatic preferred) is dissolved in a 2 per cent to 5 per cent solution of alkali. The concentration depends upon the nature of the alkali; a 2 per cent solution for caustic alkalies and a 5 per cent solution for borax or carbonates. Dyestuffs,

fillers, etc., are stirred well into the casein solution and then a very dilute solution of acid is added, cold, while the casein solution is kept well agitated. The precipitate is roughly pressed to remove the greater part of the water, then introduced into a mold or form fitting into an hydraulic press capable of delivering a pressure of at least 500 pounds per square inch. After the press is closed, the plattens are usually heated with low pressure steam, though the same result is obtained in the cold by holding the casein under pressure for a longer time. If heated, and if no dyes or fillers were used, the white curd will be found converted into a straw-colored or brownish mass, in from one to twelve hours, depending upon the thickness of the plate. It is then removed from the form and introduced into the formaledhyde solution to "harden."

The hardening process is essentially a reaction between casein and formaldehyde. It is therefore necessary for the formaldehyde solution to penetrate completely the casein sheet or rod, so the time of hardening will again depend upon the thickness of the piece of material, varying from about two weeks to six months. There seems to be considerable disagreement in the literature regarding the best concentration of the formaldehyde solution, Clement and Riviere [2] recommending 35 to 45 per cent, Eugen Stich,[10] not over 35 per cent, and Hans Blücher [11] claiming that no reliable statements are to be found, which last is also our conclusion. It seems to be a matter of individual adjustment to individual cases, but 35 per cent appears to be a rather high concentration. They all agree that it is important to keep close check upon the solution so as to hold the concentration constant, taking care to correct for the separation of paraformaldehyde. They also recommend care in providing adequate ventilation of the room containing the formaldehyde tanks, and the supplying of rubber gloves for the workmen. Formaldehyde, aside from disagreeable immediate effects, such as irritation of nasal passages and eyes, has a decided physiological reaction upon those too frequently exposed, taking the form of dermatitis, throat irritation, or nerve exhaustion. It is also important to keep the formaldehyde vapors away from casein material in course of manufacture or from casein in storage.

Upon completion of hardening, the material is allowed to season or dry. This also is a slow process, requiring about the same time as hardening. Moderate heat, carefully controlled, helps, but care must be exercised to apply it slowly and never allow the temperature to exceed 45° C., as otherwise the material is apt to fracture.

Another method, described by Paul W. F. G. Betts,[12] is of interest. Acid casein is dissolved in 2 per cent ammonia solution to produce about a 10 per cent solution of casein. After addition of dyes or fillers, as above, dilute formaldehyde solution in excess of that necessary to neu-

tralize the ammonia is added and the whole well stirred. In a short time it will coagulate en masse, forming a white, gelatinous mass, if dyes or colored pigments were not used. If this is allowed to stand a few hours, it becomes quite tough and spongy, so that the liquid is easily removed by pressure. This is roughly done in a suitable press, such as a screw hand press, and the semi-dry coagulum transferred to a form in an hydraulic press, as described above. Upon removal from the form it does not require further hardening with formaldehyde, but merely to be dried or seasoned.

Below is given a list of patents concerned with the production of plastic masses from casein by wet process methods. This list does not claim to be complete, but it contains a fairly representative group, from which may be gained a fair idea of the process and its modifications.

Patent Number	Patentee	Date
Ger. 127,942	Krische and Spitteler	.....
Ger. 115,681	Adolf Spitteler	July 15, 1899.
Ger. 201,214	Julius Kathe	July 14, 1905.
Ger. 216,215	" "	July 20, 1905.
Ger. 240,249	Hans Stephen	Sept. 24, 1910.
Ger. 264,567	" "	Sept. 11, 1912.
Ger. 292,282	Edmund Zillich	Sept. 6, 1913.
Fr. 425,204	Soc. An. Franc. de Chemie Indust.	June 6, 1911.
Fr. 361,896	Lebreil and Desgeorge	Nov. 9, 1905.
Ger. 240,584	" "	Dec. 12, 1909.
Aus. 46,988	" "	.....
Brit. 2,027	" "	1910.
U. S. 1,023,022	" "	Apr. 9, 1912.
U. S. 1,031,814	" "	.....
Fr. 450,635	Franz Thomas	Nov. 15, 1912.
U. S. 1,159,878	" "	Nov. 9, 1915.
U. S. 1,159,879	" "	Nov. 9, 1915.
U. S. 893,129	Paul W. F. G. Betts	July 14, 1908.
U. S. 1,360,356	Harry P. Bassett	Nov. 30, 1920.
U. S. 1,511,003	D. C. Polden	Oct. 7, 1924.
U. S. 1,112,297	L. L. T. Labbe	Sept. 29, 1914.
U. S. 1,452,086	Francis G. Maries	Apr. 17, 1923.
Brit. 169,405	" "	Dec. 7, 1920.

Ger. = German; Fr. = French; Aus. = Austrian; Brit. = British; U. S. = United States.

The statement of Pufahl [13] that wet process material is inclined to be the more brittle seems to be the consensus of opinion on the subject. Another weakness of the wet process lies in the fact that it is more involved and slower than the dry, and is thus more costly in operation. In consequence it has been almost entirely abandoned in favor of the better and more economical method.

Brief mention might be made of a possible modification of the wet process. There will be found in the literature and among the patents, an occasional mention of the preparation of plastic masses from the

coagulated curd direct. As examples we cite the patents granted L. Erasmus,[14] Victor Schulze,[15] Holland Zijde Mij,[16] Pozzi and Tandelli,[17] as typical. This modification would probably work all right, but it can never be of any great importance, because of the difficulty of keeping the moist curd and of adjusting the plastics manufacture to the supply of skim-milk, both seasonal and regional.

As mentioned above, rennet casein, rather than acid casein, is generally used for the manufacture of plastics by the dry process. It is claimed that a material of more uniform color and greater strength results. As the manufacture of rennet casein is given in detail in another chapter of this book, it will be necessary to give but a very brief statement here regarding its properties which are of importance in the manufacture of plastics.

A good rennet casein, properly prepared from fresh, sweet, skim-milk, should have an ash content of 7.5 to 8.0 per cent which analysis shows to be almost entirely lime and phosphoric acid. A lower ash indicates an acid condition of the milk; a higher ash, the presence of inorganic impurities. It should show a fat content well below 0.5 per cent; have very nearly a neutral reaction towards litmus; moisture 7.5 to 9.5 per cent; be practically odorless, tasteless and nearly white in color. It goes without saying that it should be free from any foreign matter, such as pieces of wood, metal, stone, insects, etc., as the presence of any of these clearly denotes carelessness in handling.

Unless ground at the casein factory, the casein will be supplied in the granular form in which it comes from the drier. It must be ground fine enough for all to pass at least a twenty mesh screen, and better a thirty or forty mesh screen. Two forms of grinders or pulverizers may be satisfactorily used for this purpose, a mill with a series of corrugated rolls, such as is used in flour mills, or an impact pulverizer. Porcelain-lined ball or pebble mills will also be found recommended, but if these are used, the operation must be very carefully controlled and the product constantly tested to be sure that it is kept free from grit. The presence of a small percentage of grit renders the casein fit for the production of only inferior plastic material, as it prevents obtaining a first-class polish on the finished articles. If carelessly handled, the casein may contain pieces of brick or stone, bits of wood, metal, etc. To render such casein fit for use, it is necessary that these should be picked out, a tedious and somewhat costly operation, so it is well to investigate the source of supply if possible, or secure casein only through thoroughly reputable people. As a further precaution and a check on the grinding operation, it is well to sift the ground casein before mixing.

Satisfactory results may be obtained with any type of mixing machine which thoroughly turns the powder over and over on itself, has no "dead"

spaces where the powder can lie undisturbed, and which does not beat the mixture to the production of excessive dust, as the whole operation is performed with dry, or nearly dry, powder. Blücher gives a very good treatment of "Mischvorrichtungen" in his "Plastische Massen," pp. 30 to 39. In general, certain types of dough mixers seem to give

Courtesy of The Artofex Corporation.

FIG. 1.—Dough mixer extensively used in plastics industry.

good results. The type shown in Figure 1 is quite extensively used in the industry, both in Europe and in this country. Alfred Kohner [18] has patented a device for blowing the water in the shape of steam or fine spray into the casein powder. Julius Kathe [19] also has patented a process for treating ground casein with water vapor. These devices are theoretically good, but how they work out in practice may be a different matter.

The water may vary from 20 to 42 per cent, according to Boerma [20] and Amandus Bartels,[21] to 45 per cent or above, according to a patent of the Deutsche Kunsthorn Gesellschaft.[22] With it are mixed or dissolved fillers or aniline dyes, to produce the opaque or colored effects desired, and also a plastifying agent. If neither fillers nor dyes are added, a cream-colored, translucent product results, which is known as "blonde" color. The usual fillers are dry, finely powdered pigments, such as are used in the paint industry, for white and opaque colors, while bronze powders are used to produce metallic effects, such as gold, silver, copper, etc. The aniline dyes used to impart color to translucent or transparent material should be acid colors in order to maintain their shade through the subsequent formaldehyde treatment.

The object of the plastifier seems to be primarily to assist in the conversion of the powder into a homogeneous, plastic mass, though Pufahl [13] claims a certain amount of glycerine increases the elasticity of the finished product, which is without doubt true. There are quite a number of plastifiers mentioned or recommended, glycerine,[23] alpha or beta naphthol,[24] benzoic acid, carbolic acid,[25] aniline oil, acetanilid,[26] sulfonated castor bean oil, the so-called "turkey red oil," [27] acetic acid,[28] 20 per cent ammonia,[29] etc., while under this heading in "Matières Plastiques Soies Artificielles," [2] are to be found amines and amides, both fatty and aromatic, urea substitution products, camphor and glycerine, fatty sulfonic acids, such as turkey red oil, phenolic and cresylic esters, mixtures of glycerine and ammonia, phenol and zinc chloride.

According to Bartels [30] "The finely powdered casein mass quickly soaks in the water mixture and is still powdery after mixing." During the mixing appreciable heat is generated in the powder, the so-called heat of adsorption. If the mixing is not properly done (either from the use of the wrong type of mixer or from interruption of the process before its completion) and the soft lumps formed by the first contact of the solution and the powder are not broken up promptly, this heat causes a partial plastifying to take place, with the result that these lumps become quite tough. In order to restore homogeneity to the mixture, it is necessary to sift these out, reduce them to powder and mix this with the rest of the mixed powder.

The dry powder, like the original dry granulated casein, will store well and if kept dry, will keep almost indefinitely,—depredation of rodents and insects excepted. After mixing, however, the powder should be promptly used or putrefaction will set in. According to "A. T." in an article entitled "Galalith," the powder must be used within 10 to 12 hours after mixing, or it becomes unfit for use. This time can, of course, be extended somewhat by keeping the powder in a cool place, but after

it has begun to spoil, it is difficult to work and the quality of the resulting product is questionable.

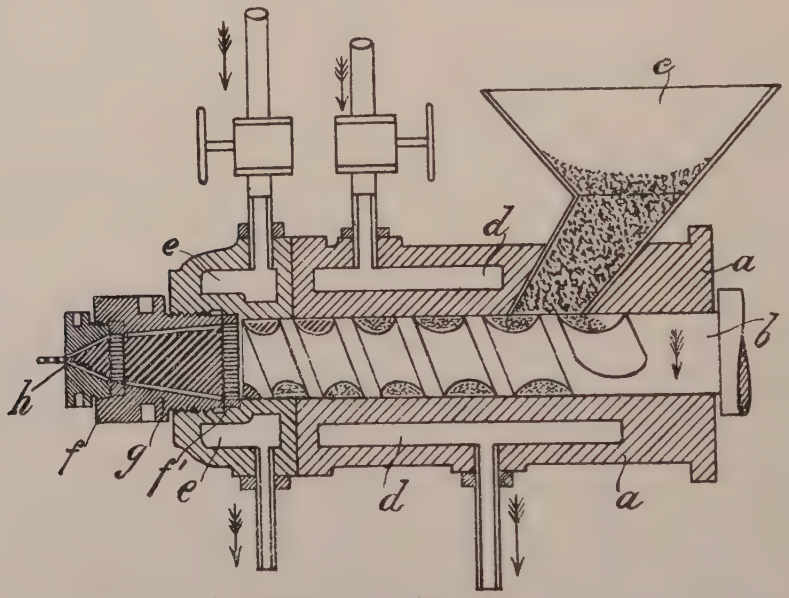
The next step in the process of manufacture is the conversion of the powder into plastic masses by the simultaneous application of heat and pressure. In order for the finished product to have strength, this should be accomplished by a rubbing or flowing of the material over or into itself. There are several ways in which this can be accomplished. "A. T." [31] suggests heated cylinder presses, having rotating screws, such as are used in the rubber industry, or pressing the powder in a heated hydraulic press into a sheet twice the thickness desired, cutting the sheet in two and repressing the halves to fill frames of the same dimensions, but half as thick. Clement and Riviere [32] and Hans Blücher [11] suggest steam heated kneading machines, which idea is also contained in patents taken out by Amandus Bartels [21] and W. Plinatus.[33] The Deutsche Kunsthorn Gesellschaft, manufacturers of the casein plastic known as "Neolith," have several patents [22,34] covering the use of heated calender rolls, such as are common in the rubber industry, for this purpose.

The machine which seems to be the most generally used for this purpose in the industry is the heated cylinder press with the rotating screw. This, as was mentioned above, is also an adaptation from the rubber industry. Patents covering such a machine, or its application to the casein plastics industry, are held by the Internationale Galalith Gesellschaft Hoff and Co.,[35] Amandus Bartels,[36] and E. H. Johnson.[37] The machine consists essentially of a heavy conveyor worm or screw, operating through a cylinder, which is jacketed for water circulation, and through a "head," which is merely a continuation of the cylinder, fitting onto it at the discharge end, and jacketed for steam circulation. A cone-shaped bushing fits onto the head, while the mouthpiece or nozzle with bore the size of the rod desired, screws into the other side of the bushing. A sieve, or breaker, fits in between the head and the bushing and a smaller breaker, between the bushing and the nozzle. Figure 2 shows such a press.

The powder is fed into the cylinder, and while kept cool by water circulated through the jacket, is conveyed by the screw to the heated head. Here it piles up against the first breaker, thus generating pressure, until, through the influence of heat and pressure it is converted into a soft, plastic condition and is forced through the holes of the breaker. It continues to be heated while it is forced through the holes of the second breaker and out through the nozzle, issuing in the form of a rod of soft, hot plastic. If rods are desired as the finished product, this plastic rod is cut off in the desired length (usually about one meter), placed in cold water until thoroughly cold, then transferred to the hardening tank; if sheets are wanted the plastic rod is cut up into shorter lengths,

which are wrapped in cloth until ready to be laid out for introduction into the hydraulic presses.

There are a number of patents covering modifications of the machine just described. Bartels [38] controls the size of rods extruded by an attachment to the nozzle; Bartels and Miech [39] have an attachment which they claim permits the formation of large blocks of plastic by direct extrusion; Adolf Bähr [40] takes care of the back pressure on the screw and holds the pressure exerted on the material constant by means of an hydraulic



Courtesy of The Chemical Foundation, Inc., Owners.

FIG. 2.—Diagram of screw-press extruder. Reproduced from U. S. Pat. 1,211,526.

attachment and accumulator; Kaiser and Gardels [41] have a similar attachment and also have the screw bored to permit water circulation for cooling purposes; Lindener Eisen- und Stahlwerke Aktien Gesellschaft [42] have a gauge for indicating back pressure, when placed in the hydraulic line of Bähr; Eberhard [43] produces vari-colored effects by having three screws deliver plastic masses into a chamber from which the mixture is extruded from a single opening by means of a fourth screw; Butterfield [44] produces the same effect by having the screw shaft bored out to permit a second screw, considerably smaller, of course, to operate through it, both discharging into a common head; while in the January, 1926, number of "Revue Générale des Matières Plastiques," p. 41, there

are described variations in the head of the machine for the production of tubes and also a head carrying three breakers.

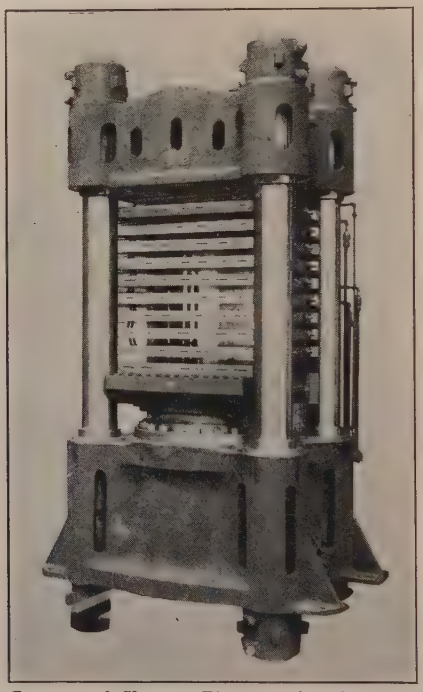
The heated cylinder press with rotating screw is an excellent machine for the continuous production of rods or tubes, the latter necessitating merely a different type of nozzle. It is possible to extrude casein plastic material through an hydraulic stuffer, such as is used in the celluloid industry, but this machine has two disadvantages over the other type. In the first place the operation is discontinuous instead of continuous, it being necessary to fill the cylinder with a certain charge, extrude, and then refill, the extrusion being of necessity interrupted during the refilling. In the second place the material is merely softened and extruded, whereas the conveyor screw rubs or smears the material into itself while exerting pressure on it. In order to produce as strong material, pieces of kneaded plastic, rather than the powder, should be loaded into the stuffer, thus entailing an additional step in the process of manufacture.

Sheets are prepared from plastic material in general by one of two methods: by pressing out the plastic in hydraulic presses until it fills frames of the proper size and thickness, or by slicing sheets of the desired thickness from blocks of plastic material of the proper size. This size may and does vary, but the major part of the sheets produced are approximately 16 by 20 inches, a size established by the Internationale Galalith Gesellschaft some years ago, so the trade is accustomed to and demands it.

The frames used in the first process are a little larger in all dimensions than the finished material (to take care of the shrinkage during drying) and heavy enough to withstand the pressure exerted upon them. The plastic material, rods from extruders, strips from rolls or pieces from kneading machines, is weighed to just fill the frame and leave a slight excess for fins, and placed on a thin metal sheet, together with, and surrounded by, the frame. A second thin metal sheet is placed over it and the whole placed between heated plattens in an hydraulic press, which is capable of exerting a pressure of about a ton to the square inch on the material. The regular standard multiple platten presses having from six to twenty openings and plattens constructed to permit heating with steam and cooling with water, have been found quite satisfactory. Figure 3 shows such a press. When all the openings have been filled, as described, the press is closed and full pressure slowly applied. After holding full pressure and heat for a certain time, this varying with the thickness of sheet being produced, the steam is turned off and cold water is circulated through the plattens without releasing the pressure. When cold, the press is opened, the sheets removed, the

metal sheets stripped out and the plastic sheet knocked out of the frame. It is now ready to be placed in the formaldehyde tank to harden.

The second method, *i.e.*, slicing sheets from a block of plastic, as is done in the celluloid industry, has a great advantage over the frame method in the fact that it is possible to produce sheets by it which are absolutely uniform in thickness. It is not applicable, however, to the production of veined or streaked effects to anything like the extent of



Courtesy of Chas. F. Elmes Engineering Works.

FIG. 3.—Multiple platten hydraulic sheet press.

the other method. This is a rather serious drawback, as such effects are much in demand by the trade.

The veined or streaked effects, such as various horn effects, tortoise shell, jade, etc., are produced by introducing into the powder, before it is fed to the extruder or run through the rolls, small pieces of previously made plastic, in the color of the streak desired. These are drawn out somewhat when the powder is converted to the plastic and the effect is completed by so laying out the plastic in the frame that when it flows out to fill the latter, the proper result is obtained, long, fine lines or

streaks in the horn effects, and short, blotchy ones in tortoise shell. Streaked rods are produced in an analogous manner, with the difference that the mixture is regulated to produce the effect desired in extrusion, there being no further operation to refine or complete it.

For introduction into the formaldehyde tanks the sheets, rods or tubes are set in suitable frames, which are designed not only to hold the material properly, but to allow as free circulation as possible of the hardening solution. The frames themselves should be easily raised or lowered, as it is necessary to test the material from time to time to note the progress of penetration of the formaldehyde. A simple method of testing consists in cutting a section of the material while it is still wet and soft from immersion in the formaldehyde solution and allowing it to dry in the air. If hardening is incomplete, lack of homogeneity is quite apparent in a few hours. The time of complete penetration will vary, as in wet process material, from about two weeks to six months,[31] depending upon the thickness of the material, and the concentration and temperature at which the formaldehyde solution is kept. The hardening process is a very critical stage in the successful manufacture of casein plastic material and requires careful supervision. This is rather difficult in view of the fact that we are ignorant of the exact nature of the reaction taking place between the casein and the formaldehyde, and have had, up to the present time, only crude methods for determining its progress. Recently, however, K. Haupt and M. Wächtler [45] have applied the optical method of Wächtler [46] to a study of "Die Homogeneität des Kasein-Kunsthorns" with rather promising results. By this method, they claim to be able to detect rings formed by variation in homogeneity through unequal absorption or diffusion. With a reliable method for detecting these differences, it will be much easier to study their causes and attempt their elimination, or at least, control. If perfect, or nearly perfect, homogeneity can be maintained through the hardening and seasoning stages, there is no doubt but the material will be relieved to a great extent from internal stresses and strains, with the result that the loss through splittage will be practically eliminated and the strength of the finished product rendered more uniform.

There are other methods for hardening the plastic to be found described in the literature. Stich [47] states that formaldehyde gas gives good results, but is impractical for large scale application. Bartels,[23] and Bartels and Miech [39] suggest mixing hexamethylenetetramine into the powder before it is converted into the plastic, and Morin [48] suggests the use of trioxymethylene in similar connection. Plinatus [33] suggests kneading hardening agents, such as aldehydes, chromium salts, tannic acid, etc., into the casein mass in vacuum; Winkler [49] also mentions chromium salts and tannic acid, while Lebreil and Desgeorge [50] suggest mixing

casein with small amounts of acetone, its homologues or derivatives. Zillich [51] claims that hydrogen peroxide substituted for formaldehyde will harden the material so it will not shrink nor wrinkle. These are all of more theoretical than practical interest, since, so far as we know, dilute aqueous solutions of formaldehyde are universally used to harden casein plastics commercially.

The seasoning consists essentially in removing by evaporation the excess moisture contained in the hardened material. The normal content of the seasoned material is 8 to 10 per cent. As stated in describing the same step in the wet process, heat assists, but care must be exercised to use only moderate heat and to apply it very slowly. The moisture in the center, particularly in the case of rods, must travel quite a distance through the material before it can escape. If the outside dries too rapidly, stresses and strains are certain to set in, which will either fracture the material immediately or produce in it a condition of unbalanced equilibrium, analogous to non-annealed glass, with the same results. The optical method of Wächtler, to which reference was made above,[46] should prove of great value in assisting to regulate the rate of drying, so as to maintain as nearly homogeneous conditions throughout the material as possible.

During the process of drying or seasoning, the material is apt to become warped to a greater or less extent. This is especially true of the sheets, which must be straightened before they can even be satisfactorily piled up for storing. This is accomplished in low pressure hydraulic presses, where the sheets are slowly heated between steam-heated plattens until flexible, the presses are closed, then cold water circulated through the plattens until the sheets are cold. When removed from the press, the sheets will be found quite flat and straight, in which shape they will remain, if properly seasoned, until climatic conditions or external pressure bends them out of shape. If the rods are required to a uniform diameter, exact to a few thousandths of an inch, or the sheets to a uniform thickness, the first must be ground and the second sanded in suitable machines.

Besides the manufacture of plastics from casein alone, or practically alone, there are a number of products in which the casein is mixed with other reacting substances, or with other plastics. A few such products are mentioned in the table on the following page.

There are numerous patents and articles to be found dealing with the manufacture, by similar processes, and from other albuminous raw materials, of plastics, analogous to the casein plastics. We will mention only two here as typical: yeast and yeast residues,[52] and soya bean.[53] There is manufactured in Germany from yeast residues a plastic known as

Casein and Phenol or Cresol.	F. G. Wiechmann.	U. S. 1,061,346	(May 13, 1913)
	Albert & Berend.	Fr. 436,720	(Apr. 3, 1912)
	Ludwig Berend.	U. S. 1,040,850	(Oct. 8, 1912)
	H. Kühl.	Ger. 280,648	(Aug. 21, 1913)
	H. Kühl.	Chem. Ind. 37, 1914, 559.	
	Comm'l Prod. Co.	Brit. 4,154	(Feb. 19, 1909)
Casein and Gelatine.	von Kagenek	Ger. 281,541	(Nov. 12, 1913)
	Hans Schwarzberg	Ger. 225,259	
	" "	Aus. 47,674	
	" " & Noa.	U. S. 864,388	(Aug. 27, 1907)
	M. Pontio.	U. S. 1,293,191	(Feb. 4, 1919)
Casein and Cellulose.	Louis Collardon	Ger. 174,877	(Feb. 17, 1904)
	" "	Fr. 359,073	(Mar. 15, 1906)
	H. E. Fauquet	Ger. 138,783	(July 25, 1901)
	H. V. Dunham	U. S. 748,709	(Jan. 5, 1904)
Casein and Cork.	Fritz Raschig	U. S. 1,170,102	(Feb. 1, 1916)
Casein and Kaolin.	Emil Merkel	Ger. 351,440	
Casein and Chalk.	Max Grosser	Ger. 371,678	
Casein and Converting agent.	Byron B. Goldsmith	Brit. 412	(Jan. 7, 1909)

"Ernolith," which has to quite an extent the appearance and properties of Galalith.

Chemistry

When we come to consider the chemical reactions involved in the transformation of dry granular casein, brittle and soluble, into the finished plastic, tough and insoluble, we are confronted with difficulty at the start. Before the reactions can be studied, the reacting substances should be considered and, at least partially, understood. The principal raw material, and the substance entering into every reaction we shall attempt to consider, is casein. What is casein?

This question we can but very imperfectly answer at the present time, and the answer is so far from satisfactory to the scientist as to be scarcely an answer at all. "Casein is the name applied to a group of related compounds found in milk, which in general react as a unit and have similar properties." This group will naturally vary with the treatment and preparation, so we can expect differences in properties between acid and rennet caseins. There are a number of other factors, such, for example, as variations in the food of the cows, differences in breed, climatic differences, etc., which quite possibly cause differences in caseins prepared by the same general process. To just what extent such differences do exist, and what their effect is on the reactions we may attempt to study or control, cannot be stated at this time. We can only state that there are abundant indications of some such variations and that we feel there is a field of research here, rich in potential possibilities for the patient, accurate worker.

In his chapter on "Properties of Colloidal Solutions," Bancroft[54] indicates the same sentiment in the words "Caseine has not been studied

so intelligently as albumin and the results are consequently less clear." He continues a little farther on in the same chapter, "Like albumin, caseine is carried into apparent solution by acids, alkalies, alkaline earths and some salts. . . . The so-called solutions are opalescent and the caseine can be removed by ultra-filtration, showing that it is not in true solution." In brief, casein is a colloid, and its reactions and characteristics, as well as the properties of products manufactured from it, will be colloidal in nature.

The first possible chemical reaction in the process occurs when the dry powdered casein is mixed with water, etc. This reaction becomes manifest by the swelling of the casein (increase in volume) and by the generation of quite appreciable heat, the so-called heat of absorption.[55, 56] A very apparent immediate result of it is seen in the toughened material of the lumps formed in the mixing and not broken up promptly. This is possibly a preliminary stage in the formation of the plastic mass. Casein possesses quite a marked tendency to absorb water, Hatschek [57] deducing from his formula for the viscosity of two liquid phases that one volume of casein binds more than eleven volumes of water, while Van Slyke and Publow [58] state that one gram of dry casein will take up 1.25 grams of water. It may be argued that this is not a true chemical reaction, but merely an absorption phenomenon. It is also argued that the dilution of concentrated sulfuric acid (1.84 +) with water, or the solution of solid caustic soda in water are not chemical reactions, despite the generation of heat. Such cases are open to doubt and hence difference of opinion, but we feel the evidence in the casein-water combination is stronger for chemical reaction than against it.

The next stage or change takes place when the casein-water-plastifier combination is subjected to heat and pressure and converted thereby into the plastic mass. This seems logically to be the continuation and completion of the casein-water reaction just mentioned, with the plastifier entering in to a more marked degree. Other factors less easily controlled or understood enter in likewise, viz., the differences in the composition or structure of the casein. Here it will be found that two caseins, apparently the same, mixed under as nearly as possible identical conditions with the same amounts of water, etc., and worked to the plastic state under the same temperature and pressure will vary radically. One may become normally plastic and work through the extruder or rolls without difficulty, while the other may exhibit either extreme; be quite soft, almost gummy, so that it is too plastic to work properly under normal pressures, or be so tough and resistant that it will work through the machine with great difficulty, if at all. These extremes can be modified so as to react almost normally by altering conditions or mixtures, but it would be quite an advantage to know exactly what to expect of a casein at the time of

purchase, i.e., have a constant, variation in which would indicate what to expect in the casein.

The most important reaction is that of the formaldehyde on the plastic in the so-called hardening. This reaction is definite and complete, yet not understood at all. One casein compound goes into the hardening bath: another compound, with entirely different properties, comes out. Casein is soluble in alkalies, has great capacity for water absorption, and when made up into the plastic and dried is as brittle and metastable as non-annealed glass. The compound or compounds removed from the hardening bath are insoluble in alkalies, have much decreased capacity for water absorption, and are tough and elastic. This reaction of hardening agents, such as formaldehyde with its other commercial forms, hexamethylenetetramine and trioxymethylene, aldehydes, chromium salts, tannic acid, acetone, etc., seems to be general for albuminous substances. It will be an interesting and valuable piece of work to study the course of this reaction and show just what takes place, together with the influence of the variables, concentration, temperature, etc., upon the end product.

The seasoning process may be said to be the completion of the hardening reaction, as it is essentially the elimination of excess water and formaldehyde from the material through the influence of gentle heat. Care must be taken in the seasoning and straightening that too much moisture is not extracted, otherwise the material is apt to crack or become quite brittle. Dehydration alone does not present a very satisfactory explanation of this phenomenon, which has the appearance of unbalanced equilibrium. Through dehydration, however, it is quite possible that the composition of parts of the material may be made to change, thus disturbing the equilibrium of the whole. A moisture content of 8 to 10 per cent is considered normal.[59]

Properties and Characteristics

From its general reactions and probable chemical composition, casein plastic material may be considered a synthetic horn. It is difficult to ignite and chars rather than burns, emitting the characteristic odor of "burnt feathers," the recognized qualitative test for organic nitrogen. It is softened to some extent by moderate heat (90°-100° C.), but to nothing like the extent of nitrocellulose bodies, and it becomes rigid again much more rapidly on cooling. It is therefore possible to bend and emboss the casein plastic material to some extent, but not to mold it. To emboss buttons, for example, the previously sawed or punched blanks are introduced into a jacketed and heated mold which is in a press of either toggle or hydraulic type. The press is slowly closed, allowing sufficient time to elapse for the material to become uniformly heated through before full

pressure is applied. As soon as the press is closed, the steam is turned off and cold water is circulated through the jacket of the mold. When cold, the press is opened and the finished buttons easily removed.

It has not proved commercially profitable to mold many articles from the unhardened plastic, largely because of the expense and difficulty attendant upon putting any quantity of small diversified pieces through the hardening and seasoning processes. At least one manufacturer, the Compagnie générale d'électricité of France, however, is very successfully molding in this manner lamp shades, from dimmers to great bowls for indirect lighting. They use aluminium molds, which are strong enough to withstand the moderate pressure necessary and at the same time they are lighter to handle than steel, cheaper to make up, and when obsolete, have a higher salvage value.

It is possible to press two or more sheets of plastic, unhardened material together to form homogeneous material in vari-colored layers. The sheets are prepared in the regular fashion, then two or more are introduced into a frame of the proper thickness and repressed with joining surfaces moistened with a suspension of calcium hydroxide in water,[39] a film of moist casein,[60] a thin celluloid sheet inserted [61] or the like. The composite sheet is hardened and seasoned by regular methods. There is also a patent held by the Société Comoy-Vincent [62] for the preparation of a product from thin plaques or plates of horn and Galalith, united under combined influence of heat and pressure. They claim for their product the strength of horn combined with the beauty of Galalith, which claim appears somewhat far fetched.

The translucent material has a specific gravity of around 1.35, the opaque, somewhat higher, depending upon the quantity and nature of the filler used. The natural color is cream, which is a good base for other colors. Consequently, the material is furnished in a great range and variety of colors and color effects, from light coral and turquoise shades to sapphire, amethyst and jet black in solid colors, and various horn effects, tortoise shell, jade, cloudy amber, etc., in mottled or streaked colors. The dyes used are practically all colors fast to light, so the color effects do not change or lose their brilliancy with the passage of time or exposure to sunlight.

The almost unlimited possibilities in color production and the non-flammability of the material are the two strongest and most generally used sales talking points of the casein plastics manufacturers. Coupled with these is the fact that, practically, it is quite a tough and strong product, having a modulus of rupture of around 15,000 pounds per square inch. It is true that this is not as high as celluloid, but it is high enough for many applications where non-flammability is more important than greater strength.

It is fairly easily machined, turned, drilled, ground, etc., but inclined to be rather hard on tools, due to the fact that it is practically a non-conductor of heat, so the heat generated by friction with the cutting edge of the tool has to dissipate through the tool itself. In consequence, the tool will lose its cutting edge or dull rather quickly and allowance must be made for this. A machine in which the cutting tool requires a lot of careful adjustments each time it is placed in its holder, or a cutting tool so complicated as to be difficult to sharpen are not practical for use on this material. In general, high carbon steels seem to give the best results, but the individual application and method of application will govern this by experience. For successful drilling, a spiral drill having plenty of clearance should be used. The drill should never be crowded and, if possible, a small stream of water should play on it while operating.

From this it is very evident that in order to work casein plastic material successfully, it must be studied and worked by methods applicable to it especially. Too frequently a manufacturer will try to force it to work by methods employed for celluloid, vegetable ivory, pearl, etc., and on machines designed for these materials. The inevitable result of such a course is failure, coupled with condemnation of casein plastic material in the mind of the manufacturer. A good example of the lack of success, and worse, attendant upon such a course, is told by Glückner,[63] who relates at some length the grief experienced by the German hard rubber comb manufacturers when they first took up galalith and attempted to work it analogous to hard rubber, and on machinery entirely adapted to the latter. Very satisfactory results may be, and are, obtained with casein plastic material properly worked. The two largest manufacturers, The Internationale Galalith Gesselschaft Hoff and Co., Germany, and The Erinoid Ltd., England, publish and distribute to the interested trade pamphlets containing general working directions. Karl Wernicke,[64] and an anonymous writer [65] give some general directions for bending, glueing, polishing, etc., but the details of good working methods are not to be found in print. They are the result of experience of the real conscientious workers in the trade and are held by them as trade secrets. In reality, they are part of the workers themselves and in many instances could only partially be reduced to print. Another case where the real secret lies in the operation,—technique.

There has been to date no very good use found for the waste material, such as web of sheet left after blanks have been sawed or pocked out, turnings, shavings, etc. In the celluloid industry this is worked over to quite an extent into second grade material, but the only way casein plastics waste might be used this way is ground and mixed into fresh mixtures as filler material. Schwarzbach [66] suggests such disposal, especially where mixed with phenolic resins. Another possibility is use as a fertilizer, the

material containing a comparatively high percentage of organic nitrogen and a much lower percentage of phosphoric acid. However, in this country it is very doubtful as to whether it would sell for enough to much more than pay the expense of grinding and handling. Some disposal which would enable the trade to realize a return would be of considerable importance to the industry, as this waste is now a dead loss and amounts to as high as 40 per cent in some instances.

The most serious weakness of casein plastic material is a direct legacy from casein. The property of casein to absorb water is of considerable importance in the manufacture of this material, but this tendency, though

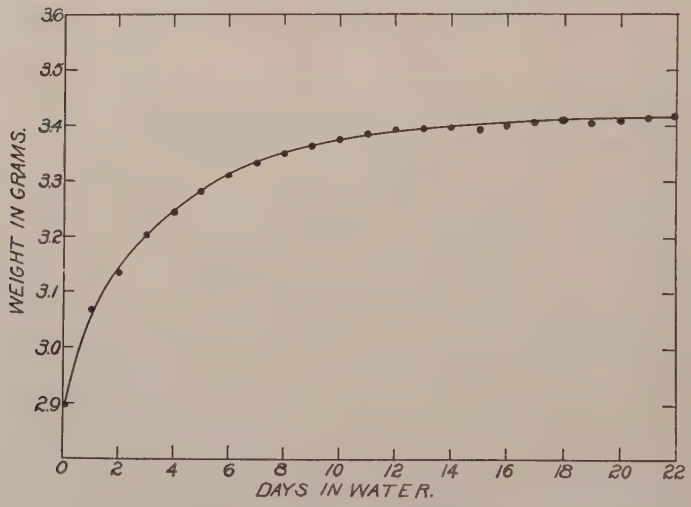


FIG. 4.—Absorption of water by casein plastic material.

checked to a very considerable extent by the hardening treatment, eliminates it from a number of fields and seriously restricts its application in others. Karl Wernicke [64] gives the following figures on water absorption, as indicated by increase in weight:—24 hours, 22.5 per cent; 5 days, 31-32 per cent; 6 weeks, 31-32 per cent. These are entirely out of proportion to any results we have obtained from similar tests and must have been worked out under extreme conditions, or on a very improperly hardened material. We show a curve, Figure 4, plotted from experimental data, on which is indicated the gain in weight of a piece of casein plastic material soaked in distilled water at room temperature for a total of 22 days. At the end of the first 24 hours a gain of about 6 per cent is shown, in 5 days about 11 per cent, and at the end of 22 days about 18 per cent. This case is typical; at the time these figures were obtained

a number of similar determinations were made and all gave practically the same results.

With the absorption of water the material softens and swells; with its evaporation it hardens and contracts. Thus there is a definite volume change, which is not good for the material, tending to make it brittle and causing fractures to occur. This property also causes the material to warp out of shape, as it is almost impossible for absorption and evaporation to take place uniformly, and lack of homogeneity causes stresses and strains which have a tendency to distort the material. This tendency is serious enough to make the material inapplicable for use in large panels (unless amply supported) and long rods. The volume change also makes it inapplicable where accurate or definite dimensions are necessary, as, for example, sliding or friction fits. On days of high humidity, it will swell sufficiently to tighten the sliding part so that it can be moved only with difficulty, if at all, while on very dry days, it will shrink enough to cause the part to fall off.

Casein plastic material is a very good electrical insulator when dry, but its hygroscopic tendencies affect this property to such an extent that it can be used practically in this connection for interior work only. A piece of this material which showed a dielectric of 700 volts per mil. when dry, dropped to 110 volts after being in moist air for seven days. Karl Wernicke [64] states that the material is a good insulator when dry, it requiring 32,000 volts to pierce a 5-mm. white sheet and 40,000 volts a black sheet of the same thickness. If these figures are reliable, they are of interest as indicating the effect of filler material upon this property, the white material containing filler and the black probably not.

The great advantage which casein plastic material has over horn and many other products, both natural and synthetic, is the ease with which a very brilliant and pleasing polish is obtained. The working directions, to which we referred above, all contain methods and formulas for obtaining this beautiful finish. In general, they are alike, recommending a rag buffing wheel for polishing large objects, and tumbling barrels for small. A finely powdered pumice stone and water is generally recommended for cutting off the outer layer with whatever scars and defects it may carry, and a beeswax, tripoli or rouge mixture for producing the final polish. If the first operation, known in the trade as "ashing," is properly done and the surface well prepared, the second is a very simple and rapid operation. This polish is fairly permanent, provided the material is not scarred and scratched by being rubbed against itself or harder materials, nor subjected to frequent handling with damp hands or to uses where it will frequently become alternately wet and dry. In the last instance, after a time, the surface will be found covered with a network of fine

surface fractures, which do not affect the strength of the material practically, but destroy the full beauty of the polish.

Another property of casein plastic material of considerable industrial importance as well as of theoretical interest is its tendency to act as a semi-permeable membrane. It allows water to penetrate freely, but not substances, such as dyestuffs dissolved in the water. This makes possible the preparation of beautiful two-color effects by a very simple and economical process. The object is made up and polished, the dyeing operation not affecting the polish, while if polished afterward, part or all of the stain would rub off. It is introduced into a dye bath, consisting of an acid aniline color dissolved in water to which a few drops of an acid, such as sulfuric, have been added, heated to 90°-100° C., and left for 3 to 10 minutes with occasional stirring. Upon removal it will be found completely dyed or stained. If a design is now cut in it, it will be found that the dye has penetrated only a few thousandths of an inch, so the design will stand out in the original color of the material before it was stained.

Applications and Possibilities

It can readily be appreciated that a material having so many decorative and manufacturing advantages is certain to find application in a number of fields. Its greatest weakness, as has been stated, is its tendency to absorb water and there is no doubt but that this rules it out of some applications, but there are many possibilities where this property is no serious objection.

The principal application at present in this country is in the manufacture of buttons, for which it is almost ideal. From large decorative dress buttons to small plain shoe buttons, it finds successful application, being more beautiful than its competitors, horn, celluloid or vegetable ivory, and of as high or higher quality at a lower cost. For dress, fancy, or novelty buttons its unlimited color combinations give it a field almost to itself; for overcoat or suit buttons its possibilities in mottled effects in different shades make it really superior to horn or vegetable ivory (although the trade will not admit this), while for small, simple buttons, such as shoe, shirt, or underwear, it can be worked advantageously and cheaply on automatic machines. For the last, rods have been principally used up to the present time, and the buttons have been polished by tumbling. Sheets are universally used for the other types. Blanks are cut from the sheets, usually by tubular saws, and are turned on lathes or engraved by special machines, or, more rarely, they are embossed. The embossed button has not proven as popular as the properly turned one for two reasons. In the first place it has a tendency to flatten out or return to its original shape if heated by a pressing iron through a

dampened cloth, and in the second place, unless quite a high-class mold is used and the embossing operation carefully performed, the button will have the appearance of the cheap composition button, and so lose caste. The larger buttons are ashed and polished on high speed rag buffers.

Buckles require about the same qualities as buttons, viz., color range to match the color of the fabric, high polish, ease in working, etc. Circular dress buckles are, of course, made up similarly to dress buttons, with the metal parts fastened on by small screws. Slots required in most rectangular belt or coat buckles may be cut by means of emery wheels or milling cutters.

Owing to the fact that this material is at the same time decorative and a good electrical insulator when kept dry, it is coming to be used considerably for interior electric fixtures. For wall switch plates it can be made up to harmonize with the color scheme of the room, thus being much more decorative than the brass plates, while at the same time, safer. Should a connection for any reason touch the plate, as frequently happens, there is no danger of a short circuit if the plate is made of casein plastic material. In Europe they are making beautiful little electric switches in various colors of this material and connecting them to the lamp by means of silken cords. These are used to a great extent by the hotels and clubs, especially on the Continent. Until it can be overcome, the tendency to warp disqualifies this material for use as large radio panels, but it can be applied to good effect for smaller panels and for some radio parts, such as grid leaks.

Through extensive advertising, two large manufacturers of fountain pens in this country have led the public to desire decorative pens in color, rather than the conventional black. Inasmuch as the ink is held in rubber or metal containers within the pen barrel so that the ink does not come into direct contact with the material of the barrel, it has proven a very good application for casein plastic material. The color range and mottled effects make it possible to please any taste, while the cost of the material and of fabricating it is low enough to make it possible to supply the pens at a very moderate price, a price considerably less than that established by the originators of this idea, who use other materials for their pens. The same holds true in the case of automatic pencils. Care must be taken, however, to so plan the mechanism as to call for no friction fits on the material, for reasons already given. The hard rubber nib carrying the pen point (this must be hard rubber as it is in contact with the ink) and the rubber or metal container for the ink must either be threaded to screw into the barrel, or, if thrust in, must be glued. Moisture of the hand will in time cause the polish to be dulled by the formation of a network of small surface fractures, but this does not in any way interfere with the usefulness of the instrument nor entirely ruin its beauty.

The most extensive application of this material in Europe is in the manufacture of combs. The most beautiful effects are possible in tortoise shell, Irish horn and the like, and it has the advantage over hard rubber in not being charged by static electricity during use, and over celluloid, in being non-flammable. In this country this application has not, up to the present time, proven possible. The comb, whether made here from domestic or foreign material, or imported made up, will not hold up through our sudden climatic changes. With the first cold, dry days of autumn it will become so brittle that if the teeth are bent down by passing the thumb along them, they will break off with a snap and fly in all directions. The European climate is more uniform, both as regards temperature and humidity, which probably accounts for the difference in the behavior of the combs.

Other applications are beads, brush backs, manicure sets, cuticle sticks, paper knives, teething rings, cigarette holders, millinery ornaments, chess men, checkers, dominos, cane and umbrella handles and novelties.

If this material can be rendered non-hygroscopic, there is no doubt but that its field of useful application will be greatly extended. It is difficult to see how this can be accomplished in the present method of manufacture, since if it is made impervious to water in the plastic, it would not be possible to harden it by immersion in formaldehyde solution. It is not, however, impossible to change the process so as to accomplish this end. One way this might be done would be the preparation of a dry powdered molding compound which, after pressing in a heated mold, would come out in the finished form, similar to Bakelite or shellac, requiring no further hardening or seasoning. Into such a powder some water-resistant material might quite conceivably be mixed.

Until some such change is made, however, the casein plastic business may very well be content to apply itself to those articles which can advantageously be made from it. And this is a very nice little business, judging by the figure of 3000 tons produced in Germany in 1925, according to an estimate in the *Paint, Oil and Drug Reporter,* and a production of 4000 to 4500 tons in France in 1924, according to Clement and Riviere in "Matières Plastiques." The business is in its infancy in this country. What it will grow into depends to a very great extent upon how it is managed and developed. The manufacturers have to be taught how to apply it to their products and shown wherein such application is of advantage to them. If this is carefully and sensibly done, there is no doubt but the product has good future prospects, as it has much to recommend it.

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Chapter 7

Casein Glue

By Frederick L. Browne

Convention demands that a discussion of casein glue begin with a reference to its great antiquity. Report has it that the craftsmen of ancient Egypt, Greece, Rome, and China employed it in their finest cabinet work. Several museums exhibit articles of furniture from those eras which the savants assert are fashioned in part with casein glue. However this may be there seems to be good reason to believe that it entered into the woodworking of the Middle Ages and continued in use in some European countries to a small extent until modern times.

Claims for the long pedigree of casein glue, whether they will stand the critical scrutiny of science or not, are at least plausible, for the raw materials have been readily available since prehistoric times. The curd of milk with nearly any alkali will yield a glue, and if lime be chosen for the alkali, the glue may be highly water resistant. The very minor part played in modern times by casein as compared with animal and vegetable (starch) glues is due no doubt partly to the demand for casein as a food and partly to certain inconvenient properties of the simple casein-lime formula.

At the present time the great bulk of the casein glue used in this country is consumed by the woodworking industries. It finds application to a lesser extent in many other fields, in some of which its usefulness has probably been appreciated for a much longer time. No doubt as the properties of casein glue become more widely known and the glue itself is still further improved the number of its industrial uses will multiply.

The literature discloses some hesitancy among scientists [1] about accepting the trade's designation of "glue" for the casein product. The terms "adhesive," "cement," and even "dope" have been suggested instead. Manufacturers have never been troubled with such philological niceties but have followed the obvious line of least resistance in selling a casein "glue" to old, established industries whose adhesives have always been known by that name. This is one case in which the business man's point of view can be justified not only by appeal to the authority of the Century Dictionary but on substantial scientific grounds as well.

Classification of Glues.

That the word "glue" was used for many centuries to apply chiefly to animal glue may be admitted, but the equally prevalent use of the verb "to glue" indicates that a property rather than a kind of matter is the fundamental idea expressed by the word and that it should apply as well to other materials whose possession of the same property makes them similarly useful. The power of forming strong jellies which adhere to the surfaces to be joined is the principal characteristic of a glue. Such power is just as prominently displayed by casein as by animal glue, although there are important differences in the manner in which gelation is brought about.

We may define a glue as a material capable of being obtained in a condition of viscous fluidity for purposes of application and having the power of forming subsequently jellies of high cohesion (the property underlying "jelly strength" and "tensile strength") and adhesion to surfaces to be joined. The appropriate consistency for obtaining a thick film on smooth surfaces which will remain intact under pressure results usually from a condition of colloidal dispersion as a lyophilic sol, the situation pertaining in both casein and animal glues. The term "cement" might properly be reserved for those materials applied in a condition of plastic, lyophobic suspension having a high "yield value," and which set to hard, stony masses largely through the formation of interlocking crystals or crystals embedded in an amorphous matrix. Typical cements have little adhesion for surfaces to which they are applied and are weak in tension, though strong in compression. The recent work of McBain and Hopkins,[1] who find that such substances as ice, sulfur, and oleic acid can be used to join surfaces under appropriate conditions, may make it necessary to add a third class of adhesives, namely, substances applied in the form of pure liquids or true solutions which congeal *in situ* as a coherent, solid mass, related no doubt in respect to its physical state to a glass.

Although most of the present day casein glues are hard and stony when dry, they are used in the state of hydrophilic sols, they form highly cohesive jellies, and they adhere strongly to the surfaces to which they are commonly applied. They are therefore correctly designated as glues.

Glues may be classified conveniently according to the manner in which gelation is brought about. A scheme so based on the physical properties of the glue instead of on the raw material from which it is made seems logical in view of the fact that it is the properties that control the usefulness of the glue in industry rather than the materials from which it is made.

1. Glues which gel by cooling of a lyophilic sol.

Hide glue and bone glue are typical of this class. They swell in cold water and "melt" on warming. When the temperature falls again they gel and the strength of the resulting jelly increases as it dries out.

2. Glues which gel by the heating of a lyophilic sol.

Albumin glues of the more common type are heated to temperatures usually in the neighborhood of 100° C. to bring about gelation. Crude blood albumin has found some commercial application as a glue and egg albumin glues have been prepared on a laboratory scale. Another type of albumin glues belongs to class 3.

3. Glues which gel without temperature change, as a result of a chemical reaction taking place in a solution or in a sol.

The most important casein glues belong to this class. The casein may be dissolved first as sodium caseinate in a solution containing calcium hydroxide. Calcium caseinate then forms by reaction between the sodium compound and the lime, and being stable in the gel form only, under the conditions prevailing, the glue slowly forms a jelly. Both animal and blood glues of this type can be prepared by the introduction of compounds whose reaction liberates formaldehyde, which in turn combines with the protein to form a compound capable of gelation.

4. Glues which gel through evaporation of solvent.

a. Glues using water as the solvent. Some casein glues, vegetable (starch) glues, "liquid" glues, mucilage, and sodium silicate are dispersions or solutions in water which gel as they dry out.

b. Glues using non-aqueous solvents. Nitrocellulose glues employ non-aqueous solvents whose evaporation leaves a jelly.

5. Glues in which gelation is brought about without the use of a solvent.

Phenol-aldehyde condensation products have been used as glues in an experimental way at least. Although they may be applied to the surfaces to be joined in solution in suitable solvents, the latter may be allowed to evaporate completely before uniting the surfaces. At any rate, the gelation is the result of a direct chemical reaction not dependent upon the presence of the solvent. Usually heat is applied to make the reaction go on more rapidly.

In considering a system of classification based upon the manner in which gelation is brought about it must be remembered that in a given glue, more than one factor may, and in fact generally does, have an influence on gelation. Thus the change of a high-grade hide glue from the sol to the gel condition is affected to some extent by loss of water through evaporation or absorption by the wood, but the outstanding factor which must be considered in using it is the temperature effect. It therefore belongs in class 1 rather than in class 4a. By suitable treatment,

however, this same hide glue might be changed into a liquid glue in which gelation depends upon loss of moisture, placing it in class 4a. Again by incorporating a formaldehyde compound a glue may be made whose gelation depends primarily upon a chemical reaction but in which both the temperature and the evaporation effects must be kept in mind. Such a glue belongs rightly in class 3. The classes in the above scheme are not separated from each other by sharp lines of demarcation but they represent the several factors upon which gelation may depend and whose predominating influence governs the use of a glue in industry.

Water-resistant Glues.

The casein glues used for woodworking are usually advertised as "waterproof," a superlative which implies an enviable state of perfection to which even casein glues have not yet attained. Some blood glues in fact are more nearly waterproof than casein glues, and among the latter there is a wide divergence in the degree to which they are affected by water. A more modest comparative term commends itself to the technical man. "Water resistant" seems to be entirely suitable.

The water resistance of a glue no doubt depends upon the effect of imbibed moisture on its strength and adhesion. All glues with the possible exception of phenol-aldehyde condensation products absorb more or less water when brought in contact with the liquid or vapor. Some of them take up enough to become fluid again. Imbibition of water decreases the strength of jellies to shear or tension, although the degree to which they are affected differs widely. Wood substance, that is the lignin and cellulose making up the essential structure of wood, is a jelly which absorbs moisture very readily and is weakened thereby, but when fully swollen still retains a large proportion of its strength when dry. The average strength in shear parallel to the grain of sugar maple is about 2450 lbs. per sq. in. when air dry and 1380 when green.[2] With jellies of animal glue the strength is much more seriously reduced by the imbibition of moisture as is illustrated by Figure 1.[3] The slope of the curve for water resistant casein glue jellies would lie between those for wood and for animal glue, moisture decreasing the strength more than it does that of wood, but not so much as that of animal glue.

Most of the glues used in woodworking are stronger than the shearing strength of wood and when properly applied are capable of giving joints in which the failure resulting from sufficiently high shearing stress takes place in the wood as much as or more than in the glue line, provided that the glue is reasonably dry. As the joints take up moisture, however, the strength of the glue decreases more rapidly than that of the wood and may go so far that the joint falls apart under the stress of the swelling of the wood, especially if a joint in plywood is in question. Glues which

retain enough strength to hold plywood joints together after wood and glue have become saturated with water are considered water resistant for woodworking purposes. A glue which under these conditions remains so strong that when stressed to failure the break takes place entirely in the wood merits the designation "waterproof."

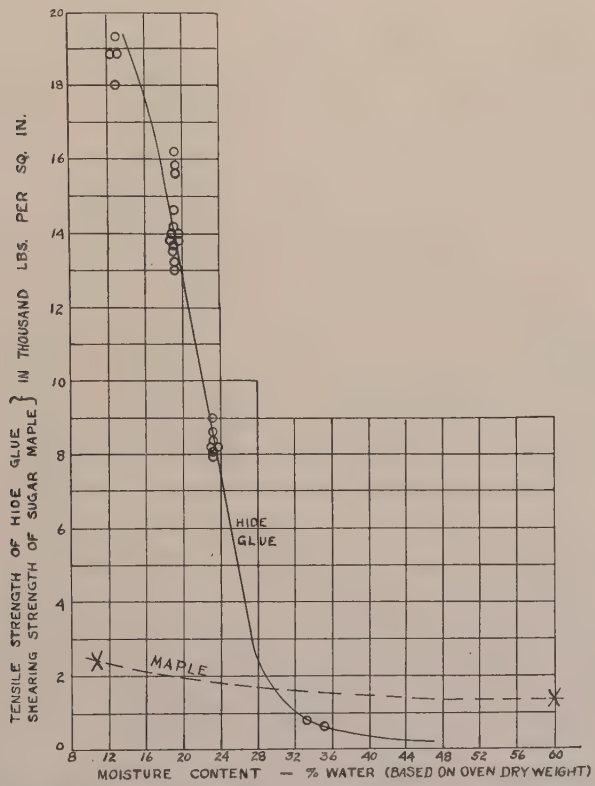

TENSILE STRENGTH OF HIDE GLUE
SHEARING STRENGTH OF SUGAR MAPLE } IN THOUSAND LBS. PER SQ. IN.
HIDE GLUE
MAPLE
MOISTURE CONTENT — % WATER (BASED ON OVEN DRY WEIGHT)

FIG. 1.—Relation between tensile strength of hide glue or shearing strength parallel to grain of sugar maple and their moisture content. (Adapted from Bateman and Town, *Ind. Eng. Chem.*, **15**, 371 (1923). Values for maple from U. S. Dept. of Agric. Bulletin 556.)

The above definition of a water-resistant glue and the implied method of measuring that property are far from satisfactory but they seem to be the only ones available at the present time. There is reason to believe that among glues which fall far short of classifying as water resistant under such a severe method of testing, there are differences in degree of resistance to moisture great enough to be of significance in their industrial

applications. A better definition of water resistance will be obtained when methods are developed for testing the strength under different moisture conditions of the glue jellies themselves, independent of the making of glued joints.

It should be noted that the property of water resistance is not inherent in the principal component of the glue but is due to the physical state of the glue jelly. Animal glues are not ordinarily water resistant, but by suitable treatment they can be made as resistant as casein glues. On the other hand, although most commercial casein glues are water resistant, there seems to be a growing tendency to employ less water-resistant types for certain purposes where exposure of the finished product to moisture is not to be expected.

The water resistant glues are found either in classes 4b and 5 or in classes 2 and 3 of the above scheme. The non-aqueous glues probably offer the best promise for developing a waterproof glue, but have been faced so far with the two obstacles of high price and difficult technic of application. With the aqueous glues, water resistance depends upon bringing about an *irreversible* gelation. In the case of untreated animal glues the change from sol to gel is reversible and the resulting glue lacks water resistance. By introducing formaldehyde in a suitable way an irreversible gelation is brought about which may yield a water-resistant glue. Blood albumin glues owe their high water resistance to the irreversible nature of the heat coagulation of proteins or to reaction with formaldehyde. Casein glues likewise may be water resistant when the gelation is irreversible.

General Description of Casein Glues

From the standpoint of marketing methods casein glues may be classified as

(a) prepared glues
(b) wet-mix glues.

The prepared glues are sold in the form of dry powders containing all necessary ingredients except water. The user adds the powder to water at room temperature in the proper proportions, employing a mechanical mixer. Most of them can be mixed ready for application even in large batches in less than 30 minutes. Once mixed, the glue must generally be used within a working day or less. Some casein glues employed in fields other than woodworking must be warmed to bring them into solution and they may not be subject to the restriction of use within the working day.

The wet-mix glues are made up as required for use from ground casein, water, and the additional chemicals called for by the formula.

Both prepared and wet-mix casein glues are used by the woodworking industries in large quantities. Each type has advantages. Wet-mix glues permit the use of certain desirable ingredients that cannot be incorporated in prepared glues very successfully. They make it possible to add the components in the most advantageous order, a matter of importance with some formulas. The casein can be allowed to swell thoroughly in water before adding the other reagents so as to bring it into solution more rapidly and uniformly. With the wet-mix glue the formula can be varied at will to meet special requirements, and in general a smaller amount of alkali can be used in making the glue. Possibly the most important reason for the wide use of wet-mix glues at the present time is the fact that the formulas commonly used (of which formulas (10) and (11) are typical) require the addition of slightly more water per pound of dry ingredient and use very inexpensive materials so that the cost per square foot of glue line may be less than that of prepared casein glues.

The prepared glues, on the other hand, require the purchase and storage of but one material instead of three or more. In mixing the prepared glue there are two components to measure out instead of four or more and it is possible to measure them by volume, dispensing with the use of a balance altogether, although such practice is extremely dangerous. When a wet-mix glue is used the ingredients must be carefully weighed out by a trustworthy operator if uniform results are to be obtained. Such a man is not always to be found in charge of the glue room of woodworking plants. The manufacturer of prepared casein glue takes the responsibility for seeing that the casein and other ingredients of the glue are of proper quality for the purpose and incorporated in the correct proportions. Since technical caseins vary widely enough to require variations in the formula in order to keep the consistency and working life of the glue uniform, and since there are several qualities of lime and of water glass on the market, this service performed by the glue manufacturer is an important advantage in favor of prepared glue.

In computing the relative costs of wet-mix and prepared casein glues per square foot of glue line it should be remembered that the wet-mix glue must be charged with the extra cost to the purchasing and traffic departments of buying and handling several raw materials instead of one, the cost of extra supervision, the extra labor cost in preparing the glue for use, and possibly a slightly higher proportion of the general factory overhead. In other words, the glue consumer using a wet-mix formula is acting as his own glue manufacturer and should charge the costs of that operation to his glue before trying to compare his glue line cost with that of a prepared glue.

Chemistry of Casein Glue

The development of casein glues has proceeded along the lines of simple empiricism with little or no attempt to apply deductively modern knowledge of the proteins, of physical chemistry, or of colloid science. Formulas embodying all the fundamental principles of present day glues have been given in books, encyclopædias of receipts, and patents for at least half a century. The formulas are all expressed in typical "cook book" style, many of them are not very useful, and most of them give inadequate instructions for mixing the glues properly. A discussion of the chemistry of casein glue at the present time can hardly be expected to prove very satisfying to the chemist because no serious attempt has been made to obtain the necessary data, in spite of the fact that a scientific investigation of the subject offers an enticing prospect not only for the practical man looking for better glues but for the scientist interested in the chemistry of the proteins and the problems of colloid chemistry.

The voluminous literature concerning the chemistry of casein must be applied cautiously to the problem of casein glue for the reason that the concentrations involved are very unusual and the casein itself is undergoing profound alterations. There is present usually only 3 or 4 grams of water per gram of casein, the water contains 5 or 10 per cent of dissolved alkalies and salts and has a pH exceeding 12. In solutions of such high alkalinity the casein hydrolyzes, as is evidenced by the strong odor of ammonia, so that by the time the glue has set to a jelly much if not all of the casein has been split into its primary cleavage products, the composition of which is still less well understood than that of casein. The condition in casein glue thus bears a strong analogy to animal glue in which hydrolysis of the glue continues as long as it is kept at the temperatures necessary for working. In both cases the ability of the glue to form a strong jelly decreases as the hydrolysis goes on, at least beyond a certain point.

For the purpose of discussing their chemical composition it is desirable to divide casein glues into (a) class 4a glues forming reversible jellies by evaporation and (b) class 3 glues which gel spontaneously, forming irreversible jellies. The former are used to a certain extent at the present time for perhaps a large number of practical purposes, but the total amount consumed is undoubtedly much less than of the latter. They are not very highly water resistant. The woodworking industries, which consume the great bulk of the casein glue produced, generally use glues of class 3. For the most part they are highly water resistant, although for certain purposes the water resistance may be sacrificed very largely in order to secure some other desirable property.

In the remainder of this discussion of the chemistry of casein glues

the custom will be followed of speaking of "solutions" of casein and of the products formed by the action of alkalies on casein as "caseinates," although the propriety of such terminology has not passed without challenge.[4] Even the most vigorous champions of the "chemical" theory ascribe the high viscosity of protein "solutions" to the presence of complex aggregates of protein and water molecules.[5] A recent view considers that "stoichiometric" compounds are formed between casein and alkalies in the region of pH up to 10.5 but that the much larger combination taking place beyond pH 10.5 is an adsorption reaction.[6] The use of the prevalent styles in nomenclature for this chapter does not imply the acceptance of the "chemical" point of view by the author.

The use of the term "viscosity" in speaking of casein glues may also be open to criticism. Bingham [7] has shown that the consistency of nitrocellulose solutions is a very complex property which will require more than two parameters for its description. A study of the characteristics of flow of casein glues may show them to be equally complex.

Glues Giving Reversible Jellies.

Solutions of casein in alkalies containing enough of the protein to give a suitable viscosity can be used as glues. By varying the proportion of casein to water or by changing the ratio of alkali to casein at the same water content, the viscosity can be altered at will from a thin, "watery" solution to the consistency of a very thick animal glue or of molasses. This makes it possible to prepare a casein glue which will have a given viscosity with the use of a comparatively small concentration of the protein, or one containing a large proportion of solids without being too thick to flow readily, as may be demanded by the conditions under which it is to be used. Casein glues of this kind belong to type 4a in the above system of classification; that is, they yield jellies when the water is allowed to evaporate, but the jellies are reversible, absorbing water again upon exposure to it to such an extent that they go back into solution. They are capable of giving glued joints comparing favorably in strength with those made with animal glue, but they are not water resistant, at least as the woodworker uses that term.

The alkali most commonly employed, although it may be introduced indirectly, is sodium hydroxide. Ammonium hydroxide could also be used and has certain advantages when it is desired either to obtain a high viscosity with as small a concentration of casein as possible or to minimize the hydrolysis of the protein. If we start with the same or similar caseins (all formulas given in this chapter are based on the use of casein imported from Argentina), glues of very much the same consistency can be made by any of the following formulas:

(1)
Casein	100 grams	
Water	300	
Sodium hydroxide	8	(0.2 gram equivalent)

(2)
Casein	100 grams	
Water	600	
Sodium hydroxide	4	(0.1 gram equivalent)

(3)
Casein	100 grams	
Water	600	
Ammonium hydroxide	13 cc. "aqua ammonia" containing 28-29 per cent NH_3	(0.2 gram equivalent)

In these and the remaining formulas in this chapter, the brackets are used to indicate the order in which the components are mixed. Thus in formula (1) the casein is first allowed to swell by soaking it in the water and the sodium hydroxide added later. The best practice is to use a small portion of the 300 grams of water indicated for dissolving the sodium hydroxide before adding it to the casein pulp.

It should be noted that formulas (2) and (3) contain only half as much casein per unit of volume as formula (1) although the consistency is approximately the same. In applying glues, the "spreading rate," that is, the area of joint surface covered by a unit quantity of glue solution, depends very largely upon the consistency of the glue, hence it is evident that (1) will require the use of more casein per unit area of joint surface than (2) or (3), other conditions remaining the same. Since the consistency of the glue in certain cases may have to be kept within definite limits determined by the mechanical operations of the process, this relation between consistency and the amount or kind of alkali used may often be used to advantage to control the quantity of casein (or of prepared glue) required to glue a given area of joint surface.

In making up these glues, the best procedure is to weigh out the casein in the mixing vessel, add the required amount of water, stir the mixture until each particle of casein has become moistened, and then let it stand until the casein has become completely swollen. The time required for this will vary somewhat with the fineness of grinding of the casein and the temperature at which the curd was dried, but with commercial casein of good quality it should not exceed 5 or 10 minutes. The alkali is then added and the mixture stirred thoroughly until the casein is dissolved. With formula (1) solution will generally be complete in less than 10 minutes, but with formulas (2) and (3) a half to one hour may be necessary. If the mixture is warmed slightly or if warm water

is used to soak the casein, solution may be brought about within a few minutes.

Where it is not convenient to heat the mixture, a glue which is essentially formula (3) may be made up rapidly as follows:

(4)			
	Casein	100 grams	
	Water	600	
	Sodium hydroxide	8	(0.2 gram equivalent)
	Ammonium chloride	10.7	(0.2 gram equivalent)

After soaking the casein in the water, it is brought into solution quickly by adding sodium hydroxide. The solution will then be very thin. On adding the ammonium chloride, which reacts promptly with the sodium hydroxide to form ammonium hydroxide and sodium chloride, the solution thickens rapidly and acquires a viscosity suitable for use as a glue.

All of these formulas have the disadvantage of being adapted for use as wet-mix glues only. The exceedingly hygroscopic character of sodium hydroxide makes it difficult to store a casein glue containing it without danger of deterioration. It is an easy matter, however, to introduce the alkali in an indirect manner so that the casein can be mixed with all of the necessary ingredients except water, giving a dry powder which is easily handled and stored, requiring only the addition of water to prepare the glue for use. One way to do this is to replace the sodium hydroxide in formula (1) or (2) with chemically equivalent amounts of calcium hydroxide and a substance which, when dissolved in water, will react with it to form sodium hydroxide. Any convenient sodium salt of an acid whose calcium salt is relatively insoluble may be used for the purpose, provided that it is not hygroscopic and will not react with the lime or the casein as long as the mixture is kept dry. A general formula for glues similar in most important respects to (1) may be written as follows:

(5)			
	Casein	100 grams	
	Calcium hydroxide	7.4	(0.2 gram equivalent)
	Sodium X	0.2 gram equivalent	

Here X is an anion with which Ca^{++} forms a precipitate. Sodium X may be, for example: sodium carbonate, sodium oxalate, sodium tartrate, sodium citrate, sodium salicylate, sodium phosphate, sodium fluoride, sodium sulfite, sodium arsenate, sodium arsenite, sodium stannate, etc. To prepare the glue for use the dry powder is added to water in such proportion as to give 300 grams of water per 100 grams of casein.

Glues of formula (5) differ in one important respect from (1), namely, when mixed with water ready for use they remain fluid only for 15 to 20 hours, after which they form irreversible jellies. Appar-

ently the precipitate of CaX_2 reacts in part with the sodium caseinate to form enough calcium caseinate to bring about gelation, or else sodium caseinate on adsorbing the calcium compound acquires the property of gelation. Such glues may have a certain degree of water resistance, but not enough to classify them as water resistant from the woodworker's point of view.

Formula (5) must not be confused with formula (8). In formula (5) the amount of calcium hydroxide does not exceed chemical equivalence to the amount of sodium X. When the glue is added to water the calcium hydroxide is changed, presumably completely, to calcium X, leaving no free lime in the mixture.

Another method of introducing sodium hydroxide conveniently into prepared casein glue consists in the employment of sodium salts which are stable when dry but which hydrolyze freely when dissolved in water. When the mixture of casein and such a salt is added to water, the salt on dissolving hydrolyzes to an extent depending upon the dissociation constant of the resulting weak acid. The casein reacts with the sodium hydroxide liberated to form sodium caseinate, whereupon further hydrolysis of the salt takes place. Equilibrium is finally reached when such a distribution of the sodium hydroxide between the casein and the weak acid is reached that both are in equilibrium with a solution of the same pH. The amount of sodium caseinate will be greater the larger the amount of the salt added and the smaller the dissociation constant of the weak acid. Among the salts which have been suggested for the purpose are: borax, sodium carbonate, trisodium phosphate, sodium fluoride, sodium sulfite, sodium arsenate, sodium arsenite, sodium tungstate, sodium stannate, and the sodium salts of many weak organic acids. The proportion of such materials required to bring the casein into solution naturally varies widely. For example glues were made up in accordance with the following formula:

(6)

Casein	100 grams
Water	600
Hydrolyzable salt	y

in which y represents the least quantity of the hydrolyzable salt found necessary to bring the casein into solution satisfactorily. The value of y for various salts is given in the table on page 181.

The minimum amount of sodium hydroxide required to dissolve the same casein under similar conditions was about 3.95 grams (0.099 gram equivalents). With such small proportions of alkali the casein dissolves rather slowly at room temperature, but if the mixture is warmed to 60° C. solution can be brought about in a few minutes. In the case of sodium fluoride it is necessary to apply heat to dissolve the casein at all.

Salt	y in		Dissociation Constant of Resulting Acid
	Grams	Gram Equiv.	
Borax, $Na_2B_4O_7.10\ H_2O$	14.7	0.077	5×10^{-10}
Trisodium phosphate, $Na_3PO_4.12\ H_2O$	12.3	0.097	
Sodium stannate, $Na_2SnO_3.3\ H_2O$	20	0.15	4×10^{-10}
Sodium carbonate, Na_2CO_3	16	0.30	3×10^{-7}
Sodium arsenate, $Na_3AsO_4.12\ H_2O$	30	0.21	5×10^{-9}
Sodium fluoride, NaF	64	1.5	

Formula (6) is written as a wet-mix glue, that is, the casein is first soaked in water before adding the sodium salt. Ready prepared glues could be made by mixing the casein with any of the salts mentioned (except sodium fluoride), the mixture to be added to water as the glue is needed for use. However, it would be advisable to use considerably larger proportions of the salts if the glue is to be mixed at room temperatures. It is also necessary to grind the casein (or its mixture with the salt) to a finer degree of subdivision than most commercial caseins. On the other hand, too fine grinding is to be avoided because it involves danger of formation of lumps of partly dissolved casein when the glue is mixed with water. Casein that will pass 60 to 80 mesh screen is suitable for prepared glues.

The hydrolyzable salts have another important advantage over sodium hydroxide as ingredients of a casein glue in that the properties of the glue are not altered greatly by small changes in the proportion of salt employed. With formula (2), for example, a slight increase in the amount of sodium hydroxide will reduce the viscosity of the glue enormously, while there is danger of rather rapid hydrolysis of the casein if much more caustic soda is employed than called for by formula (1). Because of the buffer action of the hydrolyzable salts, however, the amount used can be varied within wide limits without endangering hydrolysis or altering the viscosity too much.

The important relation existing between the amount and kind of alkali used and the viscosity of the casein solution, or the amount of water necessary to produce a glue of given consistency, has been pointed out by Zoller.[8] Figure 2 gives his results for the relation between the viscosity of a 9 per cent solution of sodium caseinate in four different alkalies and the pH of the solutions determined with the hydrogen electrode. The reasons for the wide variation in the proportion of water

used in formulas (1), (2) and (3) will be apparent at once. The viscosity is a maximum in solutions having a pH of 9.2, falling off again at higher values of pH. With ammonium hydroxide, however, the decline from the maximum is almost negligible. Above a pH of about 11, however, a region is reached in which the viscosity remains fairly constant. Zoller believes that the flattening of the curves is intimately connected with the hydrolysis of the casein to its primary cleavage products. In all solutions in which the pH exceeds about 10.5 it is well established that the casein rapidly undergoes a profound modification whereby am-

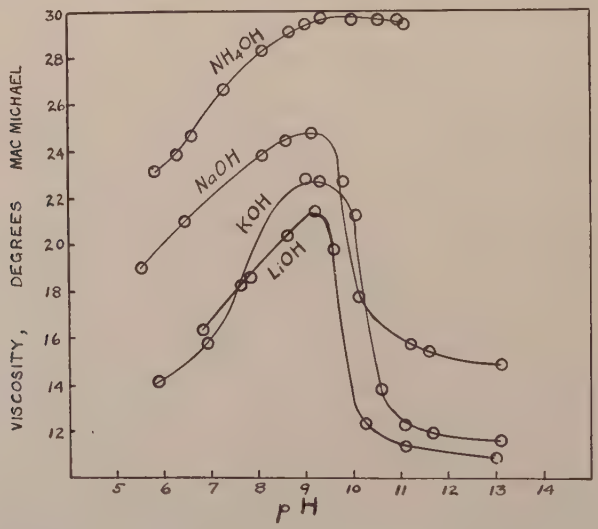


FIG. 2.—Viscosity of 9 per cent casein solutions in alkalies at varying pH.

monia, sulfides, phosphates and albumoses are split off. When the alkali chosen for solution of the casein is ammonium hydroxide, the splitting of the casein molecule may be hindered by the "mass action" effect of the ammonia.

Figure 3, likewise taken from Zoller, shows the corresponding curves for solutions of casein in several hydrolyzable salts. In considering this graph it should be remembered that a much larger amount of sodium fluoride will be required, for example, than of trisodium phosphate to produce a pH of say 9. But with all of the salts except borax a maximum of viscosity, within the limits of experimental error, is exhibited at pH 9.2, followed by a rapid decrease in viscosity for higher values of pH. With borax, the maximum occurs at pH 8.1.

Zoller suggests that the peculiar behavior of borax is due to the forma-

tion of a complex between the boric acid resulting on hydrolysis of the borax and the polyhydroxyl groups of the casein molecule, giving rise to an increased dissociation of the boric acid, just as do glycerol, mannitol, and various sugars. The preparation of acylated products of casein indicates that it may contain free hydroxyl groups.

In selecting alkalies or hydrolyzable salts and working out the formulas for casein glues these viscosity relations should be kept in mind. A thick glue which will remain in place between the surfaces to be joined in spite of a high pressure can be obtained either by decreasing

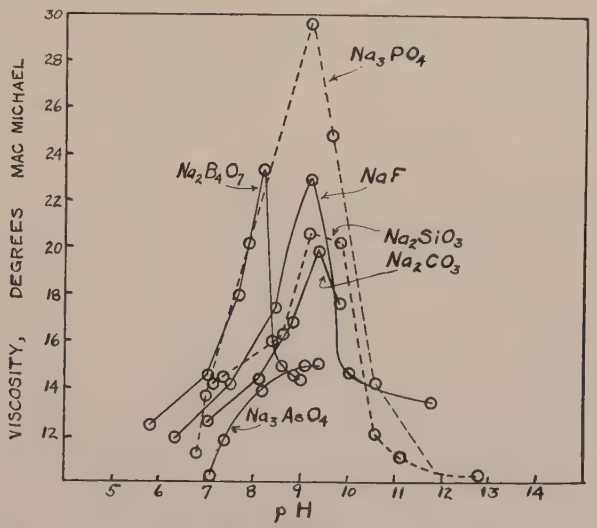


FIG. 3.—Viscosity of 9 per cent casein solutions in hydrolyzing salts at varying pH.

the proportion of water to casein or by selecting the alkali producing substance and using it in the right proportion to obtain a maximum viscosity with a high water content. On the other hand, if a glue is needed that will have a maximum fluidity and yet introduce as much casein as possible into the joint, it will be best to work with an alkaline substance and in a region in which the viscosity is at a minimum.

Casein glues of the reversible jelly type remain in a workable condition for a relatively long time after they have once been mixed. They cannot be kept safely in the liquid condition indefinitely, however, because they may be subject to two disintegrating influences, namely bacterial decomposition and hydrolysis. The former can be prevented by the introduction of antiseptics such as beta naphthol or thymol, but the latter offers greater difficulties. The products formed in the first stages

of the hydrolysis of casein are obviously high in adhesive value because the fact that all of the water-resistant casein glues are strongly alkaline and evolve ammonia freely indicates that the casein has been more or less seriously attacked, yet such glues are capable of giving very satisfactory joints. It is not until the hydrolysis has proceeded to its later stages that the glue begins to deteriorate in value as an adhesive. The rapidity of hydrolysis increases as the alkalinity is increased, especially so at the higher values of pH, but glues in which the pH does not greatly exceed 10 can be kept in a workable condition at room temperature for many days. It may be assumed safely that as long as the viscosity of the glue does not decrease materially from the value it had during the first few hours after preparation, hydrolysis has not gone far enough to impair the adhesive power of the glue.

Glues Giving Irreversible Jellies.

The glues discussed so far are of relatively minor importance industrially. They have been described in some detail for two reasons, first there is reason to believe that they will find wider employment in the future as their possibilities become more generally recognized, and secondly since the glues yielding irreversible jellies may be regarded as derived from these more simple types by introducing certain modifications it has served the purpose of clearer exposition to devote some space to their consideration.

There are two well-known ways of making casein jellies irreversible, namely, by treatment with formaldehyde and by changing the alkali caseinate to a caseinate of a heavy metal or an alkaline earth metal, usually calcium caseinate. If formaldehyde is introduced in casein glue in sufficient quantity the glue gels too rapidly to be useful. For this reason its use has been attempted so far only in those cases where the casein can be applied as a solution in an alkali and treated with the formaldehyde subsequently. It may be mentioned in passing that a similar difficulty in using formaldehyde to make animal glue water resistant has been overcome recently and it is by no means impossible that casein glues containing this reagent may be developed in the future.

Calcium caseinate has formed the basis of all commercial water-resistant casein glues so far, partly because lime is cheaper than any other hydroxide and still more because it happens to have just the right solubility in water. Calcium chloride added to a solution of casein precipitates the protein instantly as a curd, undoubtedly because the highly soluble salt reacts so rapidly with the casein that the formation of a jelly cannot take place. The limited solubility of calcium hydroxide makes the calcium available for reaction much more slowly so that gelation requires some time. If calcium salts less soluble than the hydroxide but

not too slightly soluble are used, calcium tartrate, for example, gelation under the same conditions of temperature, concentration, and pH takes place but requires a longer time. Irreversible jellies can be produced also with barium hydroxide, but the hydroxides of the other common heavy metals are all too nearly insoluble to serve as coagulants for the casein.

The maximum amount of alkali entering into combination with casein to form caseinates is ordinarily stated to be about 180×10^{-5} gram equivalents per gram.[9] This would be 6.7 grams of $Ca(OH)_2$ per 100 grams of casein. In order to make a casein glue with sufficient water resistance to withstand the Government acceptance test for water-resistant glue it is necessary to introduce several times this amount of calcium hydroxide (exclusive of the $Ca(OH)_2$ necessary to combine with ingredients other than casein). However, Hoffman and Gortner [6] have shown that casein in the region of pH beyond 10.5 adsorbs far larger quantities of alkalies than it unites with "stoichiometrically" in the region between the isoelectric point and pH 10.5. It seems probable, therefore, that the development of maximum water resistance in casein glue requires not only conversion of the sodium caseinate to calcium caseinate, but the adsorption of considerable amounts of calcium compounds in addition. On the other hand, the fact that glues made in accordance with formula (5) form irreversible jellies indicates the possibility that the gelation may be due more to the adsorption of calcium compounds as such than to the "insolubility" of calcium caseinate. It hardly seems probable that calcium caseinate is so much less soluble than calcium oxalate that it is able to remove enough Ca^{++} to convert the sodium caseinate into the calcium compound by a metathesis.

Formula (1) can be made water resistant by adding calcium hydroxide, but in order to obtain a satisfactory working life (the period of time elapsing between preparation for application and gelation of the glue) it is advisable to increase the proportion of sodium hydroxide used. The following proportions are suitable:

(7)

Casein	100 grams	
Water	200	
Sodium hydroxide	11	(0.275 gram equivalent)
Water	50	
Calcium hydroxide	20	(0.54 gram equivalent)

The glue can be mixed most quickly by soaking the casein in part of the water and then adding the caustic soda dissolved in the remainder of the water. The mixture should then be stirred thoroughly until the casein is completely dissolved. After adding the lime and stirring for a

few minutes the glue is ready for use and will remain workable for roughly 6 or 7 hours. It is capable of giving joints in woodworking of excellent dry strength and very good water resistance.

When ammonium hydroxide is used as the alkali in place of sodium hydroxide it is found, as would be expected, that between 1½ and 2 times as much water must be used in order to obtain a glue of the same viscosity. Such glues are capable of giving wood joints of good dry strength and water resistance and would therefore be very economical to use. But unfortunately the working life is inconveniently short unless the amount of lime employed is decreased so much as to sacrifice water resistance. The reason for the short working life lies in the low pH values in such glues in consequence of the weak basic properties of ammonium hydroxide. Where an inexpensive glue of moderate water resistance is required, or where a water-resistant glue of short working life could be employed such glues may well be considered.

For reasons which have already been mentioned, formula (7) is not satisfactory for making prepared casein glues. Formula (5) may be taken as the starting point for developing water-resistant prepared casein glues. By increasing the content of calcium hydroxide considerably above that necessary for reaction with the compound sodium X the water resistance is improved very materially, but at the same time the working life is cut down to about two hours. To extend the working life it is necessary to make a more alkaline glue by increasing the proportions of both lime and sodium X. A suitable formula is:

(8)

Casein	100 grams	
Calcium hydroxide	30	(0.81 gram equivalent)
Sodium X	0.275 gram equivalent	

The three dry ingredients can be ground together, packed, shipped, and stored until the glue is needed for use. The powder is merely added to water in such proportions as to give about 300 grams of water per 100 grams of casein and stirred thoroughly until the casein dissolves. Such a glue will have a working life of about 6 or 7 hours and is capable of giving strong joints with excellent water resistance.

The viscosity of these casein glues can be altered through wide limits by varying the proportions of water used, a matter which must be considered very carefully with relation to the conditions under which the glue is to be used. The working life of the glue is also affected by the water content, being greater the larger the proportion of water employed.

Another method of altering the viscosity of casein glues of this type without changing either the amount of water or the alkalinity is by adding certain heavy metal salts such as alum or calcium chloride. Small amounts of such salts added after the glue has been prepared for application

may increase the viscosity materially without affecting the working life to any great extent. It is interesting to note that calcium chloride, for example, which will immediately precipitate the casein as a curd from sodium caseinate solutions, can be added to the lime-containing glues without exhibiting any coagulating effect.

In formula (8) the proportion of calcium hydroxide can be altered within very wide limits without affecting the working life materially; namely, from about 17 grams (0.46 gram equivalent) to at least 50 grams. As the proportion of lime is decreased below the lower limit stated, the working life soon begins to increase rapidly. Since 0.275 gram equivalent of $Ca(OH)_2$ is consumed in the reaction with sodium X, at the above limit only 0.185 gram equivalent is available for reaction with the casein. This is about the amount ordinarily considered necessary to combine with 100 grams of casein to form the "completely saturated" caseinate. The longer time necessary for gelation as the proportion of calcium hydroxide is decreased below this limit, therefore, may be accounted for by considering that only a fraction of the total casein present has been converted to the gel-forming calcium compound. That view is further substantiated by the fact that as the lime content is decreased below the lower limit the glue usually loses the white, turbid appearance characteristic of calcium caseinate (in milk, for example) and takes on more of the clear amber colored aspect of sodium caseinate solutions such as formula (1). The independence of the working life and the lime content above the lower limit given is to be expected because the limited solubility of calcium hydroxide would keep the amount in solution constant regardless of the amount present in the solid phase.

But the water resistance of the glue begins to be impaired by cutting down the calcium hydroxide content of formula (8) long before the limit of 17 grams is reached; in fact, if a high degree of water resistance is desired the proportion of calcium hydroxide used should not be materially less than that given in the formula. On the other hand, this amount is sufficient for the purpose, larger proportions not increasing the water resistance to any important degree.

The working life of casein glues made in accordance with formula (8) is very greatly affected by variation in the proportion of sodium X employed, as is indicated by the curve, Figure 4. Here the working life in hours is plotted as a function of the proportion of sodium X in gram equivalents per 100 grams of casein for glues made with nine different substances serving for sodium X. In addition data are given for glues made by varying the proportion of sodium hydroxide in formula (7). The amount of calcium hydroxide used in the glues from which this particular curve was plotted was 16 grams per 100 grams of casein plus the amount required to react with the sodium X. But the curve applies

equally well for a very wide range in the lime content except for the glues containing water glass which will be discussed later. The proportion of water employed was varied in such a way as to give glues of approximately the same viscosity; that is, the glues containing 0.1 gram equivalent of sodium X were prepared with 400 grams of water per 100 grams of casein while those containing 0.2 gram equivalent or more required only 300 grams of water.

It will be seen that with the exception of the casein-silicate glues the same curve serves to express reasonably well the results obtained with

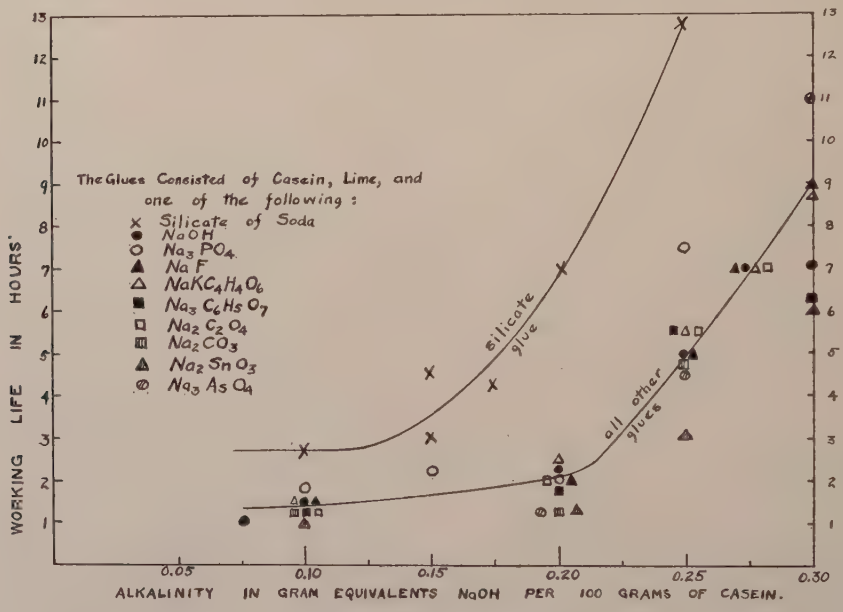


FIG. 4.—Influence of alkalinity of casein glues on their working life.

the different compounds, sodium X. Probably much closer agreement would be found if the pH of the glues was measured and plotted in place of the proportion of sodium X. The similarity in the results with so many different salts is not surprising when it is considered that, if the reaction between sodium X and calcium hydroxide proceeds in the presence of the casein as it would in the absence of the protein, all of these glues are essentially the same in composition, differing only in the insoluble precipitate of calcium X held in suspension. But the casein-silicate glue contains in addition a large amount of colloidal silica. Possibly a somewhat similar condition accounts for the exceptional values given by the sodium stannate glues. (The sodium stannate was taken

from stock without analysis and assumed to be $Na_2SnO_3 . 3H_2O$. An excess of SnO_2 over Na_2O would account for the discrepancies shown. In line with this possibility it was observed that all of these glues had a much higher viscosity than would be expected from the proportion of sodium stannate used.)

It is not clear from the meager data at hand whether the rate of gelation of casein glue depends upon the rate at which sodium caseinate is converted to calcium caseinate, the rate of adsorption of calcium compounds by calcium caseinate, or whether, the chemical reaction being quickly consummated as would ordinarily be expected, the time factor in the gelation is governed by the rate of aggregation of colloidal particles slowly building up the gel structure. Such a process as the last is to be observed in the formation of silica jellies. Water glass, somewhat diluted, may be poured into a solution containing enough acid to neutralize the alkali of the water glass. The neutralization takes place at once, as may be proved by adding a suitable indicator, but some time will be required for gelation to be accomplished.

In considering the curve, Figure 4, it should be remembered that in the presence of excess lime, all of the alkali removed from the solution by combination with the casein is presumably calcium hydroxide, leaving all of the sodium hydroxide in the solution. The point of inflection in the curve, therefore, can hardly be connected with the alkali binding capacity of the protein. The most probable explanation of the curve seems to be as follows: The working life of casein glues of this type depends upon the rate at which calcium caseinate sets to a jelly. Under the conditions of temperature and concentration concerned this would require about an hour. But we know very definitely that the casein, in consequence of the alkalinity of the solution, is undergoing a more or less profound hydrolysis, a process which would be expected to accelerate rapidly as the amount of free sodium hydroxide in the solution increased. Just as in the degradation of animal glue the jelly-forming power diminishes with increasing hydrolysis, so also with casein solutions as the casein is split into less complex cleavage products the time required for the jelly to form becomes greater.

In conformity with such a view we find that increasing the alkalinity of the glue above about 0.3 gram equivalent of sodium hydroxide per 100 grams of casein at the water content in question produces a glue which does not set to a jelly, but the viscosity, after increasing for a few hours, begins to decline again until the glue finally becomes little more viscous than water and gives unmistakable evidence of an advanced stage of hydrolysis. When sodium phosphate or sodium oxalate is used as sodium X the phosphate or oxalate ion seems to favor hydrolysis because gelation does not take place even at an alkalinity slightly less than 0.3 gram

equivalent per 100 grams of casein. Moreover, when a casein glue has once set to a jelly and is kept under such conditions that its moisture cannot evaporate, it is found that the jelly attains a maximum jelly strength in the course of time. But on further standing in the moist condition the jelly strength begins to decline until the glue becomes liquid once more and finally reaches an advanced stage of hydrolysis. The rate at which the glue passes through this cycle of changes increases with its alkalinity.

The fact that casein glues undergo hydrolysis and that the jelly finally produced in the joint probably does not consist of casein but of casein cleavage products does not detract in any way from the suitability of casein glue for the purposes to which it is commonly put in industry. The proteins of animal glue are undergoing a similar change as long as it is kept melted in the glue pot. In both cases the hydrolysis ceases as soon as the glue dries. At any rate plywood panels glued with casein glue have been found to retain a high joint strength when exposed for months to humid air and for several weeks when submerged in water. The uses to which plywood is commonly put in industry do not often involve such severe exposure to moisture.

It is assumed in this discussion that the commercial water-resistant casein glues marketed in this country conform in a general way to formula (8). It must be understood, however, that the author does not know even approximately the formulas in use by any glue manufacturer. The industry is still in the "secret process" stage of development and most glue makers guard their formulas from the public very carefully. The assumption regarding the general nature of the commercial glues is therefore based entirely upon the author's own experience with casein compositions in the laboratory and the patent literature.

The casein glue of the Middle Ages and probably of the ancients seems to have consisted of casein, water, and lime only. It is, in fact, possible to prepare a glue that is capable of giving excellent dry strength and water resistance as follows:

(9)

Casein	100 grams	
Water	300	
Calcium hydroxide	16	(0.43 gram equivalent)

The casein must be fairly finely ground and must be allowed to soak thoroughly before adding the lime in order that solution may take place as readily as possible. Once mixed, the glue must be used without delay because the working life is limited to 10 to 45 minutes, depending largely upon the character of the casein used. No doubt it was this short working life that prevented the development of the industrial uses

of casein glue for many centuries. Nevertheless during the war emergency, before more practicable casein glues became readily available in this country, at least one factory making plywood for aircraft found it possible to use such glues in their operations. Recently Dunham [10] has found it possible to extend the working life of this formula and make it suitable for a prepared glue by introducing animal glue.

Figure 4 shows that casein glues made up in accordance with formula (8) using water glass for sodium X differ radically from all the others. A much longer working life is exhibited in a glue of given alkalinity, or expressed in another way, much less sodium hydroxide need be introduced in order to attain a given working life. A second important difference lies in the manner in which variation in the lime content affects the working life. With the casein-silicate glues there is a considerable range of lime content (above that necessary to react with the Na_2SiO_3 of the water glass) in which the working life decreases as the amount of calcium hydroxide increases. With the other glues this region of inverse proportionality between lime content and working life is very much shorter.

Casein-silicate glues may be prepared in accordance with the following formula: [11]

(10)

Casein	100 grams	
Water	250	
Calcium hydroxide	20 to 30	(0.54 to 0.81 gram equivalent)
Water	100	
Water glass	70	(containing about 0.2 gram equivalent Na_2O)

It is important that the lime be added to the casein suspension before the sodium silicate.

Several glues were prepared in accordance with formula (10) except that the amount of calcium hydroxide was varied from 9 to 100 grams per 100 grams of casein. The working life of the glues was found to be:

$Ca(OH)_2$ per 100 Grams Casein in		Working Life in Hours
Grams	Gram Equivalents	
9	0.24	48
12	0.32	20
15	0.41	16
22	0.60	11
27	0.73	4
35	0.95	3
60	1.62	1¾
100	2.70	2¼

The outstanding difference in the characteristics of formula (10) and formula (8) must be attributed to the presence of the colloidal silica introduced with the water glass. The silicate of soda most commonly used for making casein glue has a silica-soda ratio of 3 to 3.25 and a density of about 40° Bé. A typical analysis might be 8.9 per cent Na_2O, 29.0 per cent SiO_2. We may consider this to be roughly 17.6 per cent Na_2SiO_3 with 20.3 per cent colloidal SiO_2. If we consider that the Na_2SiO_3 reacts with $Ca(OH)_2$ forming $CaSiO_3$, formula (10) contains 18 grams of colloidal silica per 100 grams of casein, presumably in a very highly dispersed condition. It is not surprising that the presence of this silica retards the formation of the casein jelly and prolongs the working life of the glue. In a similar way Hamill [12] finds that animal glue containing formaldehyde does not gel as rapidly in the presence of clay (for coated paper work) as in its absence.

The casein-silicate glue is very satisfactory as a wet-mix glue. Attempts have been made to prepare it on a dry basis by using a very carefully dehydrated water glass or water glass to which water soluble gums have been added before dehydration. Such a glue is said to have been marketed in this country for a time, but there is reason to believe that the makers are now using a different formula. The difficulty lies in the fact that the moisture content of the sodium silicate must be kept within narrow limits. If it becomes too dry it will not dissolve sufficiently rapidly when the powder is added to water. If it becomes too moist the powder will cake badly in the container.

Formula (10) has certain advantages over formula (8). The glue is considerably less alkaline and still has about the same working life. One result is that slightly more water is used to give a glue of the required viscosity. This, taken in conjunction with the cheapness of lime and of water glass, makes it a comparatively inexpensive glue. The lower alkalinity also reduces the rate at which hydrolysis proceeds. In extensive tests since 1918 at the Forest Products Laboratory this glue has proven to be at least equal in strength and water resistance to the best commercial glues of the prepared type.

On the other hand, formula (10) has the disadvantage that it must be prepared from the raw materials just before use and in consequence requires workmen who can be relied upon to weigh out the ingredients and follow directions closely.

The addition of a small proportion of a soluble copper salt to formula (10) has been found effective in obtaining uniformly high values for water resistance. The modification is especially advantageous where it is desired to use somewhat less lime in order to prolong the working life and yet retain a very high water resistance.[13]

(11)

Casein	100 grams
Water	220
Calcium hydroxide	20 to 30
Water	100
Water glass	70
Copper chloride	2 to 3
Water	30

After the casein has been brought into solution by the lime and water glass the copper chloride solution is added slowly and with constant stirring. As the solution mixes with the glue, the green copper hydroxide can be seen to precipitate, but in a few minutes the violet color characteristics of colloidal dispersions of copper hydroxide appears. The glue is then ready for use. Another method of mixing the glue is to add the copper chloride to the water in which the casein is soaked before the addition of the lime suspension. The presence of the copper compounds seems to have little direct influence on the working life of the glue; if anything, extending it slightly. Indirectly it extends the life by permitting the use of less lime.

Miscellaneous Ingredients of Casein Glue.

To the best of the author's knowledge the methods given above include the essentials of all the ways of making practicable casein glues that have been employed so far. Undoubtedly many other compounds than those mentioned can be employed in place of the sodium X of formulas (5) and (8) and the hydrolyzable salts of formula (6), but that does not alter the typical character of the composition.

It is not to be understood, however, that the properties of the glue of formula (8) are entirely unaffected by the choice of the anion X of sodium X. On the contrary, glues made with trisodium phosphate and with sodium fluoride, for example, will exhibit striking differences which may affect some of the details of the process of dissolving the glue in water for use.

When sodium fluoride is employed the reaction with the lime seems to proceed very rapidly on adding the glue powder to water, no doubt largely because the sodium fluoride, in the very fine crystals in which it is commercially available, has a high initial rate of solution. In such a glue the casein is "cut" almost as quickly as it would be in a solution of sodium hydroxide of equivalent concentration. Moreover, the fluoride glue shows to a marked degree that peculiar property for which the only word seems to be "tackiness," but whose significance for glue purposes

is highly problematical. These facts no doubt account for the impression some [14] have gained that sodium fluoride is the *sine qua non* of prepared casein glue.

A similar glue using trisodium phosphate instead of sodium fluoride does not dissolve as quickly on mixing with water. The crystals of the commercial salt are very much larger in size than is the case with sodium fluoride and presumably have a much lower initial rate of solution. (When equivalent amounts of commercial sodium fluoride and trisodium phosphate are added to cold water under comparable conditions the former is found to dissolve completely in a very much shorter time.) The casein-phosphate glue passes through a stage of partial solution of the casein during which the mixture is very stiff and hard to stir in the mixer. It may prove economical of power to stop the mixer paddle at this point and let the mixture stand quietly for a few minutes. When finally dissolved the phosphate glue usually has less "tack" than the fluoride glue.

There are further differences between the phosphate and fluoride glues of formula (8). Figure 4 shows that at the higher ranges of alkalinity the phosphate glues have a longer working life than glues using other sodium salts, a fact which has been attributed to a more rapid hydrolysis of casein in the presence of phosphates. With 0.275 gram equivalent of trisodium phosphate or more per 100 grams of casein, the glue does not gel, but remains workable for perhaps 2 or 3 days, finally being destroyed by hydrolysis. The difficulty of telling, in the present state of knowledge of casein glue, just how far it is safe to allow the hydrolysis to proceed without endangering the strength and water resistance of the glue makes such unusually long-lived glues of less practical importance than would be the case otherwise.

For the most part the numerous salts that can be employed as sodium X in formula (8) give glues whose general behavior lies between the extremes exhibited by sodium fluoride and trisodium phosphate.

Examination of the patent files discloses that numerous substances other than those mentioned thus far have been used in casein glues, but in most cases they serve minor or entirely imaginative purposes only. A non-drying oil, such as kerosene or castor oil,[15] is often added to prepared casein glue to prevent the scattering of part of the powder as a fine dust during handling and adding to water. Some of the glues now on the market make use of this device. Alum and calcium chloride have been incorporated in glues for the purpose of increasing the viscosity.[16] The inevitable admixture of linseed oil, rosin, and sodium resinate as a matter of course was patented years ago.[17] Camphor,[18] dextrine, starch [19] and sodium chloride [20] have been employed for purposes best known to

the inventors. Prestholdt [19] claims that magnesia prolongs the working life of a casein glue of the type of formula (7).

Glycerine is sometimes added to casein glue to make the dry glue film more flexible. No doubt other non-volatile liquids have a similar effect.

Hexamethylenetetramine [21] and formaldehyde [22] are mentioned in old patents as agents for coagulating the casein and making the glue water resistant. The formulas given are not useful because of short working life, but they may prove to be the forerunners of commercial casein glues of an entirely different type from those now in use.

Finally mention should be made of a very old patent to Majert [23] in 1896 for a process of making casein soluble by treating the dry casein with gaseous ammonia to make the ammonium caseinate. Use might be made of the method for introducing ammonium hydroxide as the alkali for a prepared glue, but it would not be useful for lime-containing glues for reasons that have already been given.

Influence of Grade of Raw Materials on Properties of Glue

Type of Casein.

A very thorough investigation of the suitability of caseins of different type for glue making was made at the Forest Products Laboratory. Over 300 samples of casein prepared by all of the commercial methods, many of them under accurately recorded manufacturing conditions, were analyzed [24] for moisture, fat, ash, and nitrogen content and for "acidity" (an empirical measure of the alkali required to render the casein solution just alkaline to phenolphthalein), and the properties of glues of three different types made with these caseins studied.[25]

The methods of analysis employed were criticized and certain modifications suggested by the Dairy Division of the Bureau of Animal Industry.[26] They also developed a borax solubility test for casein.[27]

Unfortunately the work of the Forest Products Laboratory and of the Dairy Division on methods of testing caseins and the correlating of the results with the suitability of the caseins for glue making ceased abruptly with the end of the war emergency, leaving this aspect of the glue situation in a most unsatisfactory state. Glue can be made successfully with commercial caseins of any type from grain curd to rennet, but the different caseins cannot be used indiscriminately because slight, but important changes in the glue formula sometimes must be made when changing from one kind of casein to another. The analytical methods so far developed do not tell with as much certainty as could be desired exactly what modification will have to be made in a given glue formula

to get the desired result with a particular sample of casein. No doubt the best testing methods will be found to be physical rather than chemical in nature, but such physical tests as have been proposed so far, the borax solubility, for example, have not been correlated with the glue making properties of the casein.

Of the analytical characteristics so far studied the ash content seems to be most closely related to the behavior of the casein in glue making. The higher the ash content of the casein the larger is the amount of water required to give a glue of the same consistency. Figure 5 shows

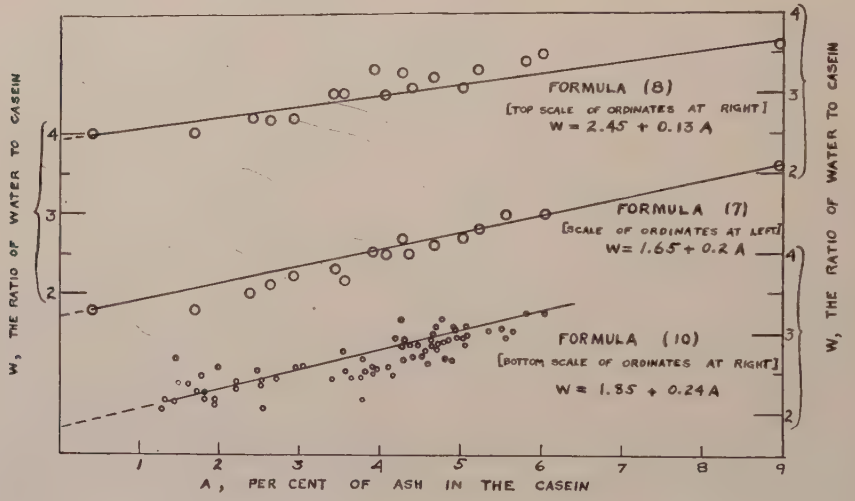

W, THE RATIO OF WATER TO CASEIN
FORMULA (8)
[TOP SCALE OF ORDINATES AT RIGHT]
W = 2.45 + 0.13 A
FORMULA (7)
[SCALE OF ORDINATES AT LEFT]
W = 1.65 + 0.2 A
FORMULA (10)
[BOTTOM SCALE OF ORDINATES AT RIGHT]
W = 1.85 + 0.24 A
W, THE RATIO OF WATER TO CASEIN
A, PER CENT OF ASH IN THE CASEIN

FIG. 5.—Influence of ash content of casein on ratio of water to casein for glues of medium viscosity. (From Butterman, *J. Ind. Eng. Chem.*, **12**, 141 (1920), and data from the Forest Products Laboratory.)

typical results obtained with a number of caseins in three different formulas corresponding closely to (10), (7), and (8). In each case the ratio of water to casein required to give a glue of an arbitrarily chosen consistency is plotted against the ash content of the casein. The curves show a simple linear relation between the two variables expressible by the equation

$$W = mA + b$$

where W and A are the water ratio and ash content respectively and m and b constants characteristic of the glue formula used.

The ash content was determined by simple ignition of the casein without adding a non-volatile base to "fix" the organic phosphorus of the low

ash caseins as recommended by Shaw.[26] If the latter procedure had been followed the straight line relationship between ash content and water requirement would no longer hold in the region of low ash caseins. Probably the CaO content of the casein is the fundamental factor governing its water requirement rather than the total ash content.

As far as the evidence so far obtained goes the method by which the casein is manufactured seems to be without much influence on the glue making properties of the casein except in so far as it affects the ash content. Of course overheating of the curd during drying may reduce very greatly the speed at which the casein dissolves, but no important influence on its other glue making characteristics has been demonstrated as yet. Zoller[8] has shown that the viscosity of casein solutions is very seriously affected by heating the curd during the manufacturing process. It might be expected, therefore, that caseins made by the "cooked curd" process would require a higher water ratio to give a glue of standard consistency than other types of casein not heated during manufacture. No doubt such an influence is to be observed in non-lime containing casein glues, but it does not seem to be so important in the glues of formulas (7) to (11). It is possible that the reason for this lies in the higher alkalinity of these types of casein glue, in which the casein is undergoing alkaline hydrolysis. For this reason the borax solubility test proposed by Zoller is not as significant as a basis of judging the merits of casein for glue purposes as might be supposed.

The variation in the water requirement of different caseins must be taken into consideration by the user of a wet-mix casein glue formula. Unless he has available a supply of casein of uniform water requirement or can obtain it by blending caseins of high and low water requirement, he must be prepared to make a suitable alteration in his formula with each new shipment of casein. When a prepared casein glue is purchased the burden of making such adjustments is thrown upon its manufacturer. The maker of a prepared glue of the type of formula (8) can use caseins of varying ash content and yet keep his product uniform in respect to viscosity for a given ratio of water to dry glue by the simple expedient of varying the amount of lime used to suit the water requirement of the casein. Thus for a high ash content casein taking more than the average amount of water a larger proportion of lime would be used so that on weighing out a given amount of dry glue a smaller amount of casein is added to the same volume of water.

The ash content of the casein also has a bearing on the working life of the glue. The following typical results were obtained with caseins of different ash content made into glue according to formula (10):

Type of Casein	Ash Content Per cent	Ratio Water to Casein	Consistency of Glue	Life in Hours
Grain curd	1.28	2.3	Medium —	12
Lactic acid	1.43	2.4	Medium —	11
Lactic acid	1.70	2.3	Medium	16
Lactic acid	2.51	2.5	Medium	10
Lactic acid	2.62	2.5	Medium +	6½
Sulfuric acid	2.64	2.5	Medium —	14
Grain curd	2.70	2.5	Medium	9
Sulfuric acid	4.29	2.7	Medium	6½
Sulfuric acid	5.06	2.9	Medium	6
Sulfuric acid	6.03	3.1	Stiff —	4½
Rennet	8.59	3.7	Medium +	5

The relation between working life and ash content is not as well defined as that between consistency and ash content, probably in part because of the crude methods by which the working life has been measured, and also very largely because certain slight variations in the technic of mixing formula (10) may affect the life to some extent.

Grade of Lime.

The grade of the lime used in making casein glue seems to be without important influence on the properties of the product provided that the amount of lime taken is calculated on the basis of its $Ca(OH)_2$ content. The Forest Products Laboratory has made an extensive study of the influence of the quality of lime on glues made in accordance with formula (10), using grades varying from very high calcium limes to products containing almost as much magnesium as calcium. No differences in the chief characteristics of the resulting glues were found when they were used in amounts proportional to the $Ca(OH)_2$ content, but if a high calcium lime was replaced by an equal weight of a lime high in magnesium, the working life and water resistance of the glue were altered. Magnesia evidently does not have the same action as calcium hydroxide in casein glue, probably because of its lower solubility.

It is most convenient to use water-slaked lime for casein glue because it is already finely powdered and also because the use of quicklime would increase the amount of heat evolved on mixing the glue. Most casein glues evolve enough heat during mixing to become sensibly warmer than the temperature of the ingredients. The heat results from chemical reaction taking place in the mixture on adding it to water, from the heat of swelling of the casein, and from mechanical work performed by the stirring. Since the viscosity of casein glue decreases rapidly as the temperature is raised, it would be undesirable to contribute further to the heating of the glue by using quicklime.

The small amounts of carbonate in commercial water-slaked lime can be neglected in calculating the amount to use in a given formula.

Grade of Water Glass.

The silicate of soda commonly used for making wet-mix casein glues has a ratio of Na_2O to SiO_2 of about 1 to 3.25 and a specific gravity of about 41° Bé. Commercial water glass varies widely in composition, the products of the Philadelphia Quartz Company,[28] for example, ranging from "S" brand having a soda to silica ratio of 1 to 3.86 and containing 31.1 per cent total solids to the "B" brand with a soda to silica ratio of 1 to 1.58 and containing 62.5 per cent total solids. Obviously the grade of silicate of soda will make an important difference in the properties of the glue.

In order to make a glue of the same alkalinity made up of casein, water, lime and silicate of soda, it would be necessary to use nearly four times as much "S" brand by weight as "B" brand. But if this were done the glue containing the "S" brand silicate would contain almost five times as much colloidal silica as the glue made with "B" brand. The calculation is based on the assumption that we may regard silicate of soda for the present purpose as sodium metasilicate, Na_2SiO_3, and colloidal silica, the metasilicate reacting with part of the lime in casein glue to form $CaSiO_3$.

Referring again to Figure 4, the curve for casein-silicate glues using "B" brand silicate of soda would lie somewhere between the two curves given, while that for glues made with "S" brand would lie just a trifle above the curve drawn for casein-silicate glues. Sodium metasilicate can be obtained commercially as a fine crystalline powder, readily soluble in cold water. It could probably be used to make a glue in accordance with formula (8) which would act very much like the glues made with other suitable materials for sodium X.

Technic of Using Casein Glue for Woodworking

The water-resistant types of casein glue have been used for the most part by the woodworking industries so far, although a more thorough investigation of the merits of the less alkaline casein glues made without lime might disclose certain applications to which they could be put with advantage.

Mixing Casein Glue.

Directions for mixing prepared casein glues in preparation for application are furnished by the manufacturer and should of course be followed very closely. It is especially important to measure out the proper quantity of the powder by weight rather than by volume because the consistency of the glue may be altered to a serious extent if the proper ratio of water to glue is not obtained. In general the directions call for placing

the water in the bowl of a suitable power mixer, then adding the powder slowly with constant stirring. Usually the stirring is continued until the glue has dissolved to a homogeneous mixture in which close inspection reveals no undissolved particles of casein. With some glues, however, it is advised to add the powder to the water and stir only long enough to distribute the glue through the mass of water uniformly and then to let it stand quietly for a few minutes to permit the chemicals to dissolve and react. The mixture is then agitated to complete the solution of the casein. A glue which passes through an exceedingly "thick" stage in the course of solution can often be handled in this way to advantage.

Wet-mix glues of formula (10) or (11) are mixed as follows:[29]

"The casein and water are placed in the bowl of the mixing machine, and the paddle is made to rotate, stirring the mixture until all the water has been absorbed and all the casein moistened. In a separate container the hydrated lime is mixed with water. This mixture is stirred vigorously at first, but just before it is added to the casein it should be stirred with a gentle rotary motion, just enough to keep the lime in suspension. Pour the "milk of lime" quickly into the casein, but reject the last few cubic centimeters, because they will contain most of the grit. Start the paddle revolving again as soon as the lime has been added.

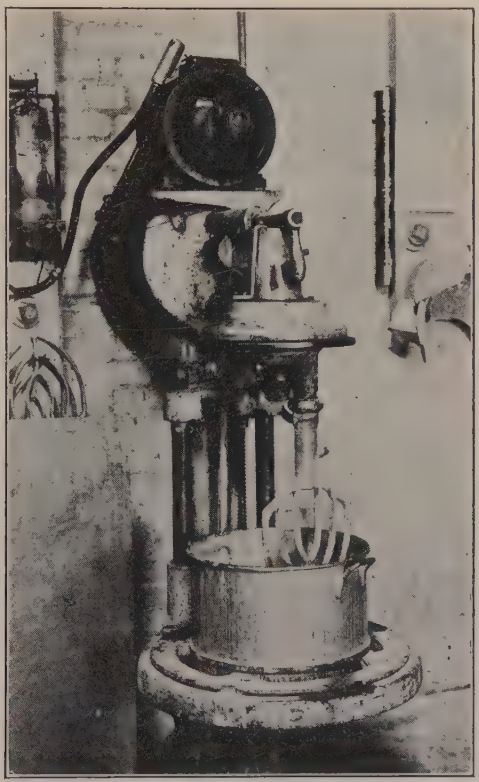
"Casein and lime act peculiarly. At first they form large, slimy lumps, which are balls of casein coated with the mucilaginous compound that lime forms with casein. These break up rapidly, becoming smaller and smaller, and finally disappear. The solution, in the meantime, is becoming thin and fluid. At this point stop the paddle and scrape the sides and bottom of the can, and then stir again. If a deposit of casein remains unacted on, it may cause lumps later.

"When about two minutes have elapsed since the lime and casein were united, it will be noticed that the glue has begun to thicken a little. The sodium silicate must be added now, or else the glue will be too thick. Disregarding lumps, if they are but few, pour in the sodium silicate. The glue will thicken momentarily, but it will soon become thin, smooth, and fluid. Interrupt the stirring to scrape the can once more, and then resume stirring until the glue is thin and free from lumps. This should not take more than 15 or 20 minutes, counting from the time the lime was added. If the glue is a little too thick, a small amount of water may now be added. If the glue is too thin, it will be necessary to start over again, using a smaller proportion of water.

"When the glue is smooth and of the right consistency, the copper salt is added. It is preferable to pour the copper solution into the glue in a thin stream, but it is possible, if the mixing is perfect, to add it all at once. Stir vigorously for about 15 minutes. The violet-colored lumps

that are formed at first by coagulation where the glue and copper solution mix are rather stubborn and hard to reduce, but stirring will break them up eventually, and a smooth, violet-colored glue will be obtained."

The consistency and working life of casein-silicate glues depend not only upon the amount of water used, but also upon the time interval between the addition of the lime and of the water glass; the longer the time elapsed the thicker the glue and the shorter the working life.

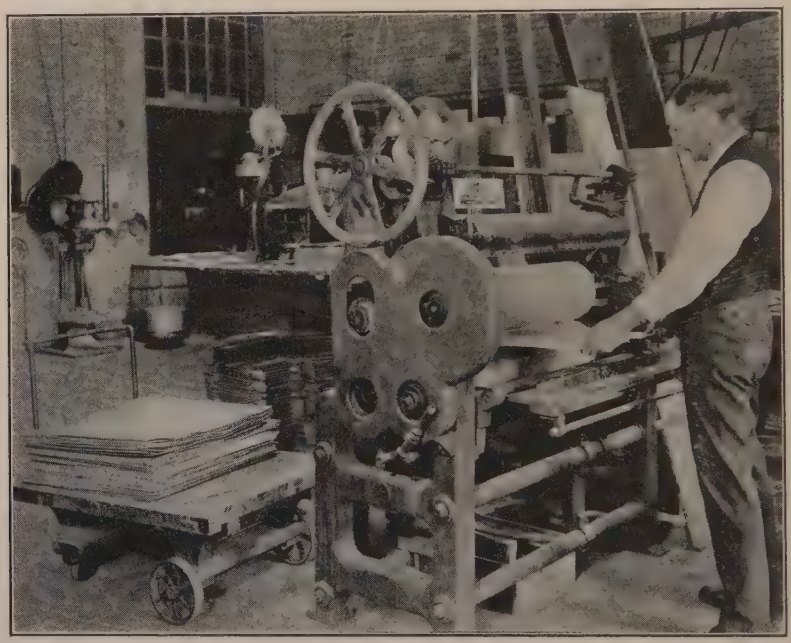


Photograph by the Forest Products Laboratory.

FIG. 6.—A casein glue mixer.

When a casein glue draws near the end of its working life the viscosity begins to increase rather rapidly. If more water is added at that time, however, the working life can be extended appreciably, but the same additional amount of water added when the glue is first prepared is much more effective in prolonging the life. On the other hand, the viscosity of the glue during its early period of usefulness may be inconveniently reduced thereby.

Proper mixing of casein glue is essential for successful use. A power mixer is required unless the amount to be mixed is only a few grams. The mixers commonly used for animal and vegetable glues are not always as suitable for use with casein glue as machines of certain other styles. The type of machine used for mixing such materials as bakers' dough will generally be found most satisfactory. The paddle should pass as close as practicable to the bottom and edges of the bowl to scrape off



Photograph by the Forest Products Laboratory.

FIG. 7.—A roll spreader applying casein glue.

any glue tending to stick to the surfaces. It is very convenient to have several speeds for the paddle and it has been found that a motion of rotation in opposite directions in two non-concentric circles gives excellent results. The bowl and paddle should be detachable for ready cleaning. The parts in contact with the glue should be made of a metal or other material not corroded by alkalies or by ammonia. Brass, copper, and aluminum are to be avoided. Figure 6 shows a type of mixer that has given satisfactory service.

Applying Casein Glue.

Casein glue can be spread by brush, but the brushes must be cleaned before the glue sets in the bristles because the glue jelly is very diffi-

cult to remove. The alkalinity is such that most brushes soon wear out. For these reasons some prefer to use wood or metal scrapers for applying the glue by hand. Its consistency is such that a tool of that kind can be employed very easily.

For large scale production the mechanical glue spreaders used for other types of glue, such as the roll spreader shown in Figure 7, are entirely satisfactory for casein glue.

From the standpoint of the technic of application casein glue takes an intermediate position between vegetable (starch) and animal glue, resembling the former more closely than the latter. Like vegetable glue it is generally used cold on cold wood and does not thicken as quickly as animal glue, but as usually prepared it flows somewhat more freely than vegetable glue. Greater latitude may be allowed in the matters of pressure and assembly time (the time elapsing between spreading the glue on the wood and applying pressure to the joint) than is possible with any given animal glue.

Relation of Pressure Used to Gluing Conditions.

In using any glue for woodworking the maximum joint strength will be attained only if a proper correlation of pressure applied to the joint and consistency of glue *at the moment of applying the pressure* is maintained. In general the more readily the glue flows the lower must be the pressure applied, and conversely, thick, jelly-like glue layers require a correspondingly high pressure to develop strong joints. That statement is based upon a very large number of empirical data obtained under carefully controlled and widely varying conditions with casein, vegetable, and animal glues and checked by observations extending over a number of years of the gluing practices in woodworking factories. If the gluing conditions are such that the film of glue in the joint at the moment of applying pressure is still very fluid and a high pressure is employed, it is found that a joint of maximum strength is not obtained, presumably because too much of the glue applied has either been squeezed out or forced too deeply into the wood. Confirming such a view, an examination of a section of the joint under the microscope generally shows an absence of an appreciable glue film between the joined surfaces. On the other hand, if the film of glue has gelled before pressure is applied to the joint, it is found that a strong joint will not be obtained unless a comparatively high pressure is applied. Bogue [30] has expressed views which do not accord with these findings and maintains that the "starved joint" is a myth, the "over-fed" joint being more dangerous. On the other hand, the conclusions of McBain and Hopkins [1] agree closely with the above principle. As a matter of fact, both the "starved joint" and the "over-fed" joint are frequently encountered in practice, although the

traditions of the glue room have tended to make the former more prevalent.

The consistency of the glue at the time of pressing depends not only upon its viscosity at the moment of applying it to the wood, but upon the changes in its condition taking place during the time necessary to assemble the pieces and apply the pressure. With animal glue, changes in consistency may take place very rapidly while assembling in consequence of its property of gelling as it cools. The pressure must therefore be adjusted in accordance with the other gluing conditions with considerable precision. The changes taking place in casein glue during this time are relatively less important because the temperature effect is eliminated and we have to deal chiefly with changes in moisture content of the glue film resulting from evaporation and from absorption of moisture from the glue by the wood. For this reason and also because of its fairly heavy consistency as compared with animal glue casein glue permits of much greater freedom from restrictions as to assembly time, pressure, and other gluing conditions.

It will be appreciated that such latitude in gluing conditions is often very important to the woodworker. In making plywood, for example, the nature of the manufacturing operations makes it necessary to build up a stack containing a large number of panels before applying the pressure. There must consequently be a great difference in the assembly time for the first and last panels in the stack, yet each will receive the same pressure.

The factors, or gluing conditions, upon which the consistency of a casein glue layer in a wood joint at the time of pressing depends will include:

The consistency of the glue used
The spreading rate of the glue (area covered by a given amount of glue solution)
The kind of wood and its temperature and moisture content
The temperature and relative humidity of the room
The assembly time
Whether "open assembly" or "closed assembly" is used.

Obviously if other conditions are the same the consistency at the time of pressing will be thicker the more viscous the glue is to begin with. Again if a low spreading rate giving a deep layer of glue is used, evaporation and absorption of moisture by the wood will produce less change in a given time than would be true for a shallow glue layer. If the wood used is of a kind that absorbs water very readily, if it has a low moisture content, and if its ability to take up water quickly is favored by raising its temperature there will be a greater thickening of the glue layer in a given length of time than under the reverse conditions. Loss

of moisture and consequent thickening of the glue layer will be increased by raising the temperature of the room, lowering its relative humidity, prolonging the assembly time, and using an "open assembly." By "open assembly" as distinguished from "closed assembly" is meant a condition in which the glue layer after application is freely exposed to the atmosphere for a considerable proportion of the assembly period instead of being covered by the second of the two surfaces to be joined.

In using casein glue, as with vegetable and animal glues, it is common practice to apply the glue to only one of the two surfaces to be joined. "Single spreading" has been found to give just as satisfactory results as "double spreading," that is, applying the glue to both surfaces, provided that a sufficient quantity of glue has been used per unit area of joint.

The conditions under which casein glues are used are so diverse that it is difficult to give very specific recommendations for the best pressure to use. Each user of the glue should determine the pressure range suitable for his work by careful tests. The results of a large number of tests at the Forest Products Laboratory indicate that good results should be obtained with pressures ranging from 150 pounds per square inch for short assembly times to about 400 pounds per square inch for a 25-minute assembly time. That statement is based upon the use of formula (10) or (11) or casein glues of similar consistency and a spreading rate of not more than 31 square centimeters per gram of glue solution. With greater spreading rates it is best to use somewhat higher pressures, and an assembly period of not more than 15 minutes. With woods having a low crushing strength care must be exercised not to use pressures high enough to injure the wood (for the strength of woods see U. S. Dept. of Agric. Bulletin 556, "Mechanical Properties of Woods Grown in the United States," Superintendent of Documents, Washington, D. C., price 10 cents).

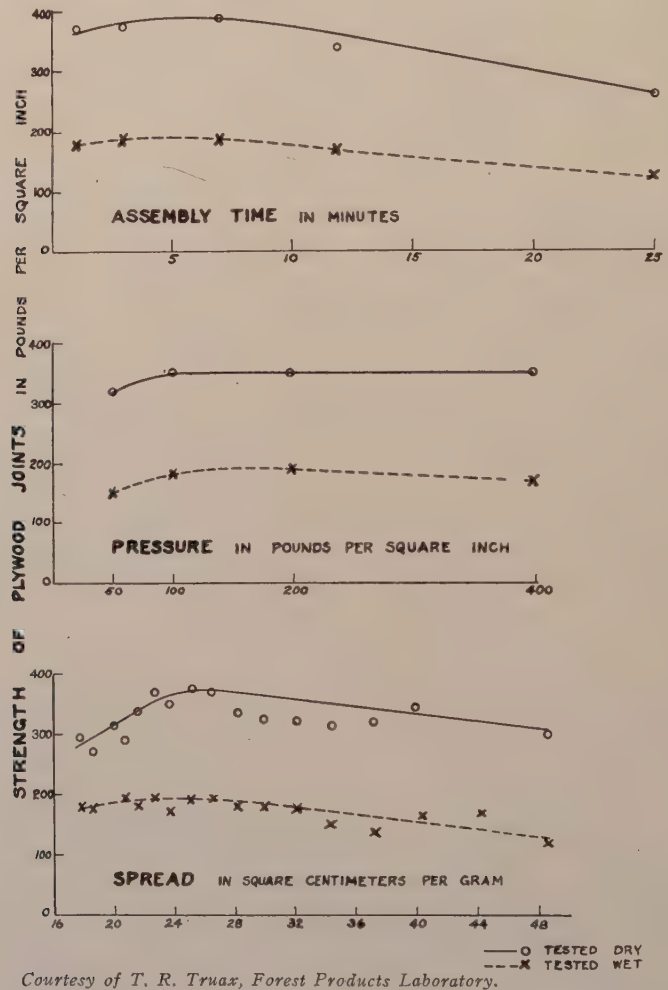
Figure 8 shows graphically the results of typical experiments illustrating the influence of the more important gluing conditions on the strength of the resulting joints. A very thorough discussion of the subject will appear in a forthcoming bulletin of the U. S. Dept. of Agric. on "The Gluing of Wood" by T. R. Truax.

Time of Pressing.

"Casein glues set very quickly and produce joints strong enough to be machined in a few hours. In spruce and woods of similar strength joints as strong as the wood can be expected within four hours from the time the pressure is applied. In hard maple, which is stronger than spruce, tests showed that about six hours were required. It is not necessary to keep the joints under pressure all this time, however. If the

pressure is maintained for at least two hours and the joints are then allowed to season without pressure, good results may be expected.

"The above statements refer to the minimum time in which good results may be obtained. In case of emergency, it might be safe to machine


STRENGTH OF PLYWOOD JOINTS IN POUNDS PER SQUARE INCH
ASSEMBLY TIME IN MINUTES
PRESSURE IN POUNDS PER SQUARE INCH
SPREAD IN SQUARE CENTIMETERS PER GRAM
O TESTED DRY
X TESTED WET

Courtesy of T. R. Truax, Forest Products Laboratory.

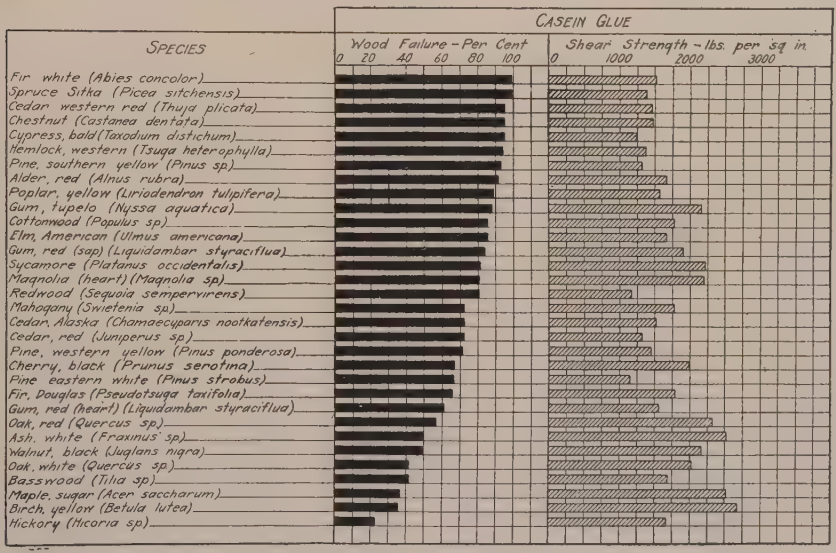
FIG. 8.—Effect of gluing conditions on the strength of casein glued joints.

wood containing casein glue joints as soon as four to six hours after pressure was applied. Under ordinary conditions this is not desirable. The best practice is to allow glue joints to season until the moisture at the glue line has dried out or distributed itself uniformly throughout

the wood. In this way sunken joints can be avoided and the tendency of the glued block to change dimension or shape after machining will be reduced." [29]

Results with Different Woods.

The use of casein glues for the gluing of a large number of woods has been thoroughly investigated by the Forest Products Laboratory. Figure 9 shows the very excellent joint strengths obtainable on a large number of woods. The tests were made by the block shear test described below. The woods are arranged in the order of the average "percentage


CASEIN GLUE
SPECIES
Wood Failure - Per Cent
0 20 40 60 80 100
Shear Strength - lbs. per sq in.
0 1000 2000 3000
Fir, white (Abies concolor)
Spruce, Sitka (Picea sitchensis)
Cedar, western red (Thuja plicata)
Chestnut (Castanea dentata)
Cypress, bald (Taxodium distichum)
Hemlock, western (Tsuga heterophylla)
Pine, southern yellow (Pinus sp.)
Alder, red (Alnus rubra)
Poplar, yellow (Liriodendron tulipifera)
Gum, tupelo (Nyssa aquatica)
Cottonwood (Populus sp.)
Elm, American (Ulmus americana)
Gum, red (sap) (Liquidambar styraciflua)
Sycamore (Platanus occidentalis)
Magnolia (heart) (Magnolia sp.)
Redwood (Sequoia sempervirens)
Mahogany (Swietenia sp.)
Cedar, Alaska (Chamaecyparis nootkatensis)
Cedar, red (Juniperus sp.)
Pine, western yellow (Pinus ponderosa)
Cherry, black (Prunus serotina)
Pine, eastern white (Pinus strobus)
Fir, Douglas (Pseudotsuga taxifolia)
Gum, red (heart) (Liquidambar styraciflua)
Oak, red (Quercus sp.)
Ash, white (Fraxinus sp.)
Walnut, black (Juglans nigra)
Oak, white (Quercus sp.)
Basswood (Tilia sp.)
Maple, sugar (Acer saccharum)
Birch, yellow (Betula lutea)
Hickory (Hicoria sp.)

Courtesy of T. R. Truax, Forest Products Laboratory.

FIG. 9.—Results of joint strength tests using casein glue on different woods.

wood failure" found in a large number of tests, using glue of formula (10) under conditions favorable to the production of strong joints. The "percentage wood failure" in a test specimen is the proportion of the area of fracture occurring in the wood rather than in the glue line. It is assumed to be a measure of how closely the joint strength obtained approaches the ideal of perfect gluing. In Figure 9 is also shown the average shearing strength at which failure of the joints took place. Inasmuch as the shearing strengths of the woods vary greatly the percentage wood failure is believed to be a better measure of how closely the joint strengths developed approach the maximum obtainable with ideal gluing conditions than the shearing strength itself.

As might be expected the stronger, more dense woods do not show as high a percentage wood failure as the woods which are weaker, but the high strength values obtained with the strong woods, usually falling very close to the shearing strength of the wood, prove the very satisfactory results that can be obtained with casein glue.

Staining of Wood by Casein Glue.

All glues containing much alkali will discolor certain woods such as maple, oak, and mahogany wherever they come in contact with them. Difficulty from this source is sometimes experienced where thin face veneers or fancy veneers with much irregular grain are applied with casein glue. No satisfactory means of avoiding the difficulty have been devised so far. Where such trouble is encountered it is advisable to use a glue of thicker consistency, insert dry cauls between the faces of adjacent panels, avoid the use of excessively high pressure, and remove the panels from the press as soon as it is safe to do so, placing them at once on stickers to dry.

Where staining has appeared it can be removed by sponging with a solution of oxalic acid or with sodium sulfite solution followed by the oxalic acid. The bleaching agents should be washed out again thoroughly as their presence in the wood might cause trouble in subsequent wood-finishing operations.

Testing Casein Glue.

The testing of casein glue from the standpoint of the establishment of specifications to serve as standards for the purchase of the glue has been confined so far to a joint strength test on maple blocks and to plywood water resistance tests. These may be reasonably adequate for the special requirements of the Army and Navy for aircraft construction but they leave much to be desired from the point of view of the general purchaser of casein glue.

In the first place both tests require expensive apparatus and skilled operators having a special knowledge of gluing technic. Few commercial testing laboratories can meet both of these requirements, to say nothing of the average factory purchasing glue for woodworking. In the second place the joint strength test is usually a test of gluing conditions rather than of the strength of the glue. Casein glue in common with the other glues used in woodworking is much stronger, when dry, than our strongest woods and the problem in using it is to carry out the gluing in such a way as to utilize as large a portion of the strength of the glue as practicable. The joint strength test, therefore, tells only what results may be expected with the glue under the exact conditions of the test; with-

out supplementary evidence it does not tell what may be expected of the glue under other gluing conditions.

Methods for obtaining the supplementary evidence in the case of casein glues have not been devised as yet. With animal glue the viscosity and jelly strength tests define fairly precisely the relative value of different glues under a variety of conditions.

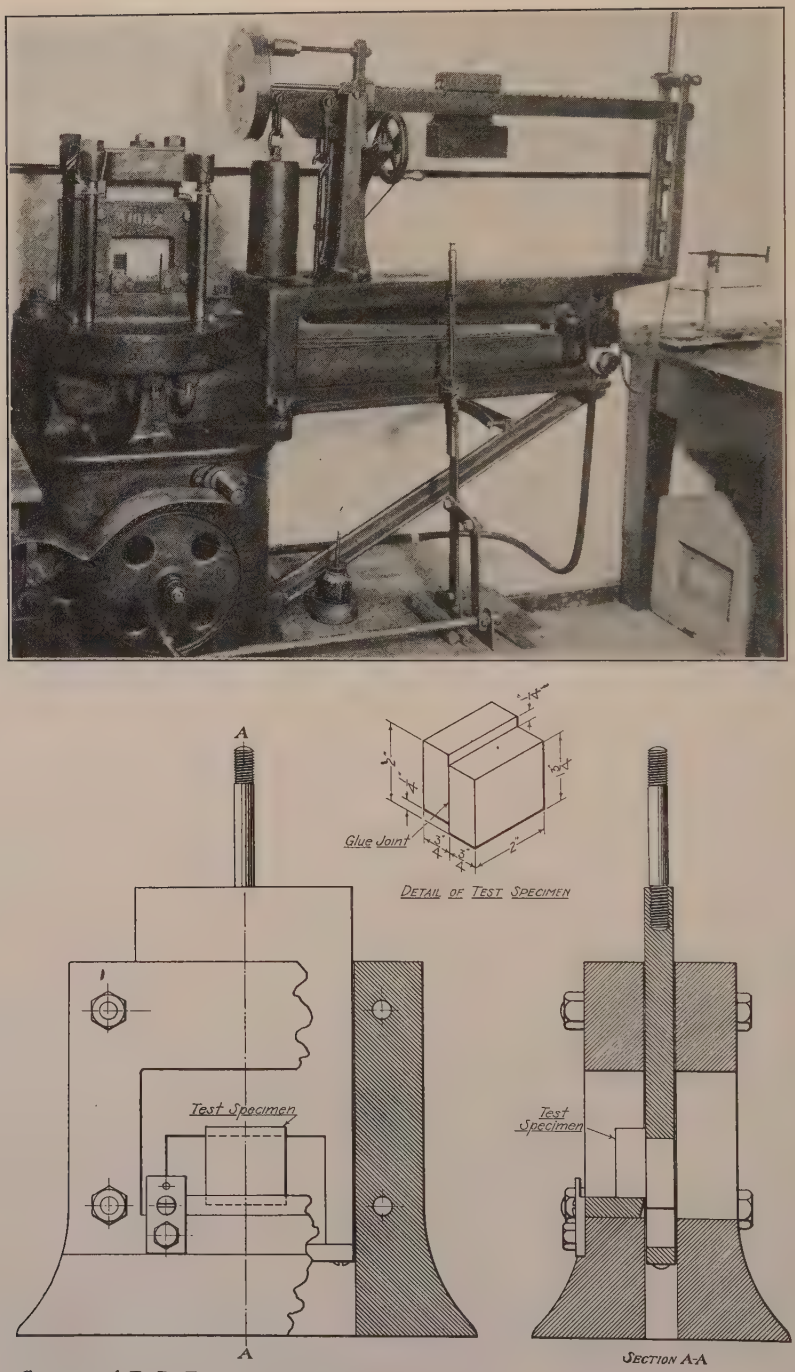
The plywood water resistance test determines very rigorously the ability of the glues to withstand severe exposure to moisture for a short time, but casein glue in the majority of its peacetime applications does not require such an exceedingly high water resistance. This test is a very severe one, admirable for selecting the best glues from the water-resistance point of view, but giving no information concerning the relative water resistance of the numerous glues which do not pass the test. There is evidence that many uses of glue exist for which a moderate degree of water resistance would be quite sufficient.

On the other hand, the present test affords information concerning the *initial* water resistance of the glue. We know that casein glues while wet are subject to hydrolysis and have good reason to suppose that there may be important differences in the susceptibility of different glues to such deterioration. We do not know how high the moisture content must be before hydrolysis becomes important nor has any convenient method been found for determining how long casein or other water resistant glues retain their water resistance.

In considering casein glue for general woodworking purposes there are often other properties than joint strength and water resistance which are of importance but for which we have no adequate test methods at the present time. A thorough revision of the methods of testing casein glue with the needs of its peacetime applications in mind would be of great value to both makers and users of the glue.

The Joint Strength Test.

The joint strength test is carried out at the Forest Products Laboratory as follows: ¾-inch hard maple lumber, free from defects and selected for straight grain and high density, is thoroughly seasoned by storage in a room at 80° F. and 30 per cent relative humidity. Just before required for a test, specimens either 2½ inches x 12 inches or 5 inches by 12 inches are cut and the surfaces to be joined are planed smooth and matched carefully. (The grain of the wood in each block runs parallel to the long dimension.) The glue, mixed exactly in accordance with the manufacturer's directions, is applied to one of the surfaces to be joined, the quantity of glue spread being determined by weighing the block before and after application. A spreading rate of about 27 square centimeters per gram of glue solution is used. The two surfaces are brought to-



Courtesy of T. R. Truax, Forest Products Laboratory.

FIG. 10.—Apparatus used in making the joint strength test.

gether, a 5- to 10-minute closed assembly time permitted to elapse, then placed in the press and a pressure of 200 pounds per square inch applied. The block is left in the press over night and then removed to the 30 per cent humidity room for seasoning for 7 to 10 days. It is then cut into 5 or 10 test specimens of the dimensions shown in Figure 10 and the specimens tested using the special shearing tool illustrated.

The load at which failure takes place, divided by the measured area of the glue line of the specimen, is recorded as the strength in shear. The specimen is examined and an estimate made of the percentage of the area of failure occurring in the wood rather than in the glue line. (Study has shown that this estimate can be made by several observers independently with a satisfactory degree of concordance.) The averages of the results with all of the specimens from at least eight test blocks are taken as representative of the glue when used under the exact conditions of the test.

The Water-Resistance Test.

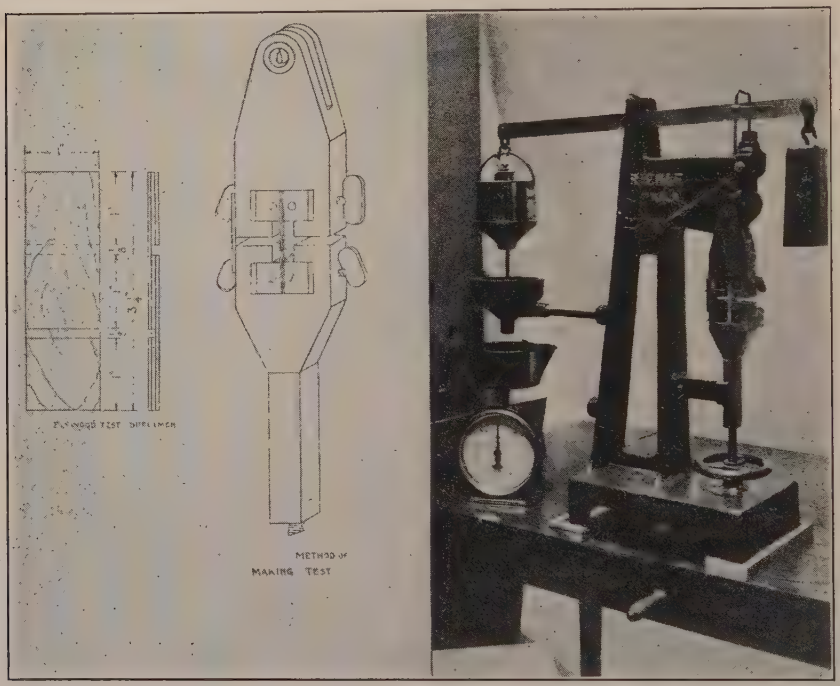
One-sixteenth-inch birch veneer, selected for straight grain and freedom from defects, is cut to 12-inch x 12-inch size and stored in a room at 80° F. and 60 per cent relative humidity until required for use. A test panel is made by gluing three of these together with the grain of the corepiece at right angles to that of the face plies (plywood construction). The glue, mixed exactly in accordance with the manufacturer's instructions, is spread on both sides of the corepiece and the amount determined by weighing the three pieces of veneer before and after application. A spreading rate of about 27 square centimeters per gram of glue solution is used. A closed assembly time of 5 to 10 minutes is allowed to elapse before applying pressure to the panel. A pressure of 200 pounds per square inch is then applied and the panel left in the press over night.

Next day the test panel, together with other similar ones, is stacked on stickers in the 60 per cent humidity room for 7 to 10 days for seasoning. Test specimens of the dimensions shown in Figure 11 are then cut, five of which are tested dry and five submerged in water for two days after which they are tested while still wet.

The test is made under tension in the cement testing machine with the special jaws illustrated. The load at failure is recorded and the percentage wood failure estimated, the averages for the five dry and five wet specimens respectively being taken as representative of the panel. At least eight such panels are made and tested and the average results for the eight panels taken as representative of the glue when used under the exact conditions of the test.

It will be appreciated that this test is an exceedingly severe one. Study has shown that the water permeates the specimens in less than two

days, causing a certain drop in strength from the value when dry. The wet strength then remains about constant for many days. If the specimens are allowed to dry out again before testing, most of the original dry strength is regained. Enormous stresses are set up in the glue line both as a result of the swelling of the glue jelly itself and because of the tendency of face ply and corepiece to oppose each other in the direction of greatest swelling. A glue to pass the test must be not only "insoluble"



Photograph by the Forest Products Laboratory.

FIG. 11.—Apparatus used for making the plywood water-resistance test.

in water (more correctly a completely irreversible jelly) but it must retain very considerable adhesive and cohesive strength in order to hold the plies together.

Panels for the water resistance test are seasoned in a room at 60 per cent relative humidity whereas blocks for the joint test are seasoned in the 30 per cent humidity room. The reason for the difference in procedure lies in the fact that where water resistance is important the glue is likely to be used in construction out of doors. The 60 per cent humidity more nearly represents such conditions than 30 per cent. When the joint test

is applied, however, the use of glue for interior construction is more likely to be in view, for which case the 30 per cent humidity is believed to be more nearly representative than 60 per cent. In general panels made from veneer seasoned at 60 per cent humidity before and after gluing will give higher values in the water-resistance test than panels made from veneer seasoned at 30 per cent.

U. S. Army Air Service Specifications.

The specifications used by the U. S. Army Air Service (No. 98-14,020C, January 15, 1925) for the purchase of casein glue are based upon the joint strength and water-resistance tests carried out essentially in the manner described. They call for an average joint strength test of 4 blocks not less than 2400 pounds per square inch with a minimum for any single block of 2000 pounds per square inch (unless the failure takes place entirely in the wood, in which case the test specimen is rejected). A plywood test of 2 panels, taking 2 sets of 12 specimens from each panel for testing dry and wet respectively, must give an average strength of not less than 250 pounds per square inch when dry and 125 pounds per square inch when wet.

Manufacturers of casein glue seem to have no difficulty in supplying a product meeting these requirements.

The markedly lower values obtained in the dry plywood test as compared with the joint test can be accounted for easily on the basis of the severe internal stresses set up in the glue as a result of the crossed grain construction of plywood and the much greater difficulty in avoiding eccentric loading in testing the thin plywood specimens. As a matter of fact the magnitude of the test values obtained in the plywood test is seriously affected by changes in the thickness of the veneer used for the corepiece or the face plies.

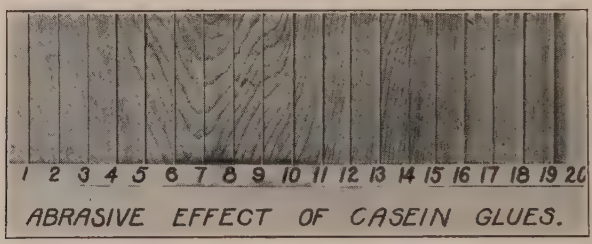
Test of Abrasive Effect on Tools.

All glues seem to have a more marked abrasive effect on tools than any of our native, commercial woods, but casein glue has sometimes given more than the usual amount of trouble from this source. Excessively thick layers of glue increase the rate of dulling of tools very markedly and it is possible that the difficulty with casein glue has been augmented in some cases by this factor. However, there is undoubtedly a wide variation in the abrasive effect of different casein glues.

A simple comparative test of the dulling power of glues can be made in the following manner: Prepare some boards of a soft wood of reasonably uniform texture, such as basswood, ¾ inch x 8 inches x 24 inches in size and one greater in number than the number of glues to be tested.

They must be worked up accurately so that the large faces are smooth, level, and parallel to each other. Now glue the pieces together one on top of the other like a layer cake, using a different glue for each glue line. It is essential that the gluing conditions be adjusted so that the glue lines are all of as nearly the same thickness as possible. This is best accomplished by having the glues at the same consistency, applying them at the same spreading rate, keeping the assembly time as nearly the same as is practicable, and using the same pressure. It is best to make a separate operation for each glue line with at least a day between them for seasoning.

When the block is completed and thoroughly seasoned, one edge is smoothed off exactly at right angles to the faces. Now with freshly



Courtesy of Don Brouse, Forest Products Laboratory.

FIG. 12.—Results of a test of the abrasive effect of 20 different casein glues.—Contrast No. 4 and No. 14.

sharpened knives, free from nicks, in the jointer, pass the edge of the block over the jointer with one face held firmly against the guide. Repeat this 30 to 50 times or until 60 to 100 linear feet of the same glue line have been passed over exactly the same parts of the jointer knives. Each glue line will then produce a nick in each jointer knife proportional in depth to the abrasive effect of the glue.

The relative depth of the nicks in one of the jointer knives can be determined by direct observation, but a simpler plan is to take another basswood board, greater in width than the thickness of the test block, square one edge of the new board with one face, and then pass this face over the jointer using the knife blades containing the nicks, keeping the squared edge firmly against the guide. A cut of considerable depth should be made or else the board passed over the knives carefully several times. The nicks in the knives produce ridges in the board. When observed with the light falling upon it from one side, the shadows produced by the ridges appear very prominently. Figure 12 is a photograph of a test made in this way.

Uses of Casein Glue

In the Woodworking Industries.

The woodworking industries consume a very large proportion of the casein glue used in this country, so large, in fact, that its other applications are of distinctly minor importance. The total amount of casein used for glue making at present is undoubtedly very great, although reliable estimates are difficult to obtain, partly because of the fact that casein finds its way into glue both through the manufacturer of prepared casein glue and through the manufacturer of wood products who buys casein to use in preparing his own wet-mix formula. It has been said, however, that the annual consumption of casein for glue making is in the neighborhood of seven million pounds.

Practically speaking, casein glue was unknown to the woodworking industries of this country before the World War. It was introduced then principally because of the necessity of producing water-resistant plywood for aircraft, none of the glues previously in common use being capable of withstanding the very severe exposures to which airplanes are subjected. (To correct a popular misconception it should be stated that casein glue was not used for airplane propellers because they had to be protected from moisture by a practically waterproof coating to prevent warping.) The experience gained during the war proved to many of its users that casein glue possesses other advantages than high water resistance and led to their adoption of it for peacetime applications, for many of which a high degree of water resistance is not altogether essential. The post-war development was no doubt greatly assisted by the prominence given the new glue by its rather spectacular war-time application which naturally overcame to a large extent the resistance which a new product exploited under more ordinary circumstances would have had to face. The permanent place of casein glue in industry seems to be assured unless some other raw material of cheaper and more stable price is found capable of yielding glue of equally desirable qualities. Future commercial development of casein glue will probably emphasize increasingly its advantages other than water resistance, although further marked improvements in the latter property may be looked for.

Plywood and veneered panel construction probably makes use of more casein glue than any other type of work, although it is very widely used in joint work of various kinds, in laminated construction, and for many special purposes. Although the future of casein glue in woodworking seems assured, in one sense it is still in an experimental stage; namely, it has not yet reached the state of technical and economic equilibrium in which it can be said that for such and such purposes in woodworking casein glue

is more satisfactory than its competitors, while for the rest it is less well adapted. At present it finds some use in nearly all woodworking industries and for nearly all varieties of work. As time goes on no doubt it will be used for certain classes of work almost exclusively and be displaced entirely from others.

The competitors of casein glue in woodworking are animal glue on the one hand and vegetable (starch) glue on the other, both well established in the industries before casein glue became important. Animal glue is higher in price, but it has in its favor the advantages of tradition and familiarity in its use on the part of the craftsmen. The wide variety of grades of animal glue and its special properties make it exceedingly flexible in the sense that the right combination of glue and gluing conditions can be worked out to fit almost any operating requirement. For joint work in which the glue must set quickly and permit of removing the work from the clamps within a few minutes animal glue is still considered superior to casein. Again, animal glue is not alkaline and therefore will not stain thin veneers of certain woods and does not make such a prominent glue line as casein.

Vegetable glue has the principal advantage over casein glue of lower price. It is widely used for plywood and veneered panels where water resistance is not a requisite.

At the present time blood glue is not a very serious competitor of casein glue for ordinary industrial uses. Blood glue is not only higher in price, but its use requires expensive hot press equipment and involves much greater labor charges. However, a few concerns are still using blood glue for special types of plywood, some of them employing unique manufacturing processes in which the hot press serves a purpose in addition to the coagulation of the glue. Where the highest possible degree of water resistance is required in plywood, blood glue may give better results than casein. On the other hand, the strength of blood glue joints under conditions of extreme dryness may not be as high as casein glue.

Casein glue has become well established in the plywood and panel trade. At the present time it appears to be making notable progress in the door manufacturing industry where it is used not only for the plywood panels but for gluing together small pieces of wood to make the cores for stiles and rails and for laying the crossbanding and face veneer.

Casein glue is also entering the automobile body industry to an important extent. It is used very largely in making sills, plywood floor boards, dashboards, and backing for upholstery. It finds application to a certain extent for joint work in making the frames for closed bodies. Many large members having curved shapes can be built up more satisfactorily by gluing together thin laminations than by steam bending or shaping a solid piece.

The possibilities for developing the uses of casein glue are very well shown by the fact that it finds a considerable use in piano manufacturing. Perhaps no woodworking industry uses gluing for a wider variety of purposes or for service requiring stronger or more reliable joints than the piano maker. At least one piano concern to the writer's knowledge has used casein glue for all of its operations for a number of years.

The Hetzer System of building construction which has been employed in a number of large buildings in Switzerland illustrates in a striking manner the important changes in the methods of using wood that may be brought about by the introduction of water-resistant casein glues. Large construction members such as roof trusses have been built up from boards by laminated construction using casein glue. The method is especially economical in the use of wood where curved members are needed.

Other Industries.

Casein glue is used to an important extent for attaching linoleum to wooden running boards of automobiles and to wood and cement floors. Apparently this use of the glue is second in importance to its use in woodworking from the point of view of the amount consumed.

A few years ago there were several manufacturers of heels for shoes using casein glue for making up sample heels and as a dipping liquid to produce a burnished, glossy appearance on the outer surfaces of the heels in the compressing operation. It seems to have been entirely displaced from this field by less expensive materials, however.

"Casein is used to a large extent in the preparation of a binder for composition cork, for which purpose it has been employed in Germany, England, Spain, and the United States. The casein is mixed with water containing borax or other solvents to which has been added glycerine or a glycerine substitute. The mixture is paddled and allowed to soak for several hours. It is then transferred to steam-jacketed kettles and melted. After an insolubilizing agent has been added it is sprayed over the granulated cork. The agglomerated mass is then packed in metal molds which are heated to the temperature of boiling water and then chilled thoroughly before the blocks of composition cork are removed.

"Another method, which employs a cold glue, produces a paler composition cork. The formula for this adhesive is:

Casein	45	pounds
Borax	7	"
Water	120	"
Glycerine	76	"

Instead of borax, other solvents such as sodium borophosphate may be used. It is important, however, that whatever solvent is chosen for the

casein it should not be of a caustic nature because caustic alkalies, including lime, have a deteriorating effect on cork.

"Arthur Biddle in U. S. Patent 1,437,487 produces a casein glue from casein and rubber latex. This use of casein with latex opens up a new field for investigation. The patent applies not only to casein adhesives, but also to the manufacture of various articles such as tiles, mats, linoleum substitutes, billiard balls, etc. In making compositions he incorporates with the casein and latex such materials as zinc oxide, china clay, fullers' earth, etc.

"Casein makes a valuable adhesive for the cementing of cork or paper discs to metal shells in the bottle cap industry. For this purpose the casein is dissolved in a mild alkali and paraformaldehyde is added in minute quantities. A slight warming of the metal of the bottle cap causes the adhesive to solidify and at the same time become insoluble. Such adhesives are used in large amounts by bottle cap manufacturers. This adhesive is used in very much the same way as an aqueous solution of blood albumin, but is very much cheaper for the purpose." [31]

Casein glue is used to some extent for pasting paper labels on tin cans and glassware. A satisfactory adhesive for this troublesome purpose seems to be afforded by a glue consisting of casein, a convenient hydrolyzable salt such as borax, and sodium resinate. The sodium resinate appears to be an essential ingredient.

Some large cigarette manufacturers employ casein glue for sealing the paper on cigarettes. They use a non-lime, prepared glue in the form of a powder which can be mixed quickly on adding to water and will not contain lumps or undissolved particles of casein. A glue requiring a minimum amount of water to obtain the consistency necessary for application is desired in order to avoid wrinkling of the paper at the seam when the glue dries and shrinks. For this reason a casein with a low ash content is used in making up the glue. One large user of casein glue for this purpose states that the cigarettes do not open up when exposed to humid conditions, a difficulty encountered with starch pastes. Apparently even the non-lime containing casein glues are appreciably more water resistant than certain other adhesives.

At the agricultural experiment stations of several universities water-resistant casein glues have been found useful for fastening the paper bags over the mature flowers of plants used for breeding studies in order to prevent uncontrolled pollination.

Numerous other alleged uses of casein glue are to be found in the literature but they have not been included because it has not been possible to verify them. Undoubtedly there are many other minor applications.

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Chapter 8

Casein in Medicine

By Peter Masucci

Casein has certain peculiar properties. As an amphoteric colloid it is capable of forming salts with either acids or bases. It is not coagulated by heat, but is clotted or coagulated by the enzyme rennin. It is unstable and hydrolyzes easily, yielding a variety of amino-acids. Because of these physical and chemical properties casein is utilized extensively in the manufacture of numerous medicinal substances. It is the chief and most important protein present in milk and because of its high food value is made the basis of many food preparations. It has also been found to be a very good vehicle for administering such remedial agents as heavy metals, tannins, alkaloids, salicylates, and iodides. In combination with these substances casein modifies markedly their physiological properties and increases their value for medicinal purposes. Acid or alkaline solutions of casein are colloidal and make excellent emulsifying agents or protective colloids. This important property is also used to advantage in the preparation of useful therapeutic substances.

Historical.—Casein in the form of sour milk has been used as a food for centuries. The souring of the milk is produced accidentally by the acid-producing bacteria normally present in milk, or is produced intentionally by the addition of specific organisms. These fermented milks are believed by many to be of value in the correction of certain gastro-intestinal disorders. There is no doubt that they are of value as growth-promoting and energy-yielding foods, regardless of any therapeutic action they may possess.

In the latter part of the nineteenth century, casein, in the form of its ammonium or sodium salts, was introduced as a patent food-stuff under various trade names. These patent and proprietary food preparations were highly exploited but their use has gradually diminished as the medical profession soon learned that they possessed no special virtues other than the food value of the casein which they contain. It was also about the latter part of the nineteenth century that casein began to be employed extensively in combination with certain drugs in order to enhance their efficacy. Its use for such purposes has somewhat dimin-

ished, as serum-albumin and gelatin have replaced it in some cases. In spite of this, the use of casein as a vehicle for other drugs still offers a good field for research.

There are no statistics available covering the quantity of casein consumed as medicinal products. In comparison with the vast amounts used industrially, the amounts used in medicine are relatively small. One American manufacturer, who uses casein in certain pharmaceutical specialties, other than foods, consumes about 10,000 pounds a year. The aggregate value of the finished products is about $150,000.

Purity of Medicinal Casein.—The purity of casein to be used in medicinal products depends largely on the nature of the product to be manufactured. In general, the casein employed in food-stuffs or in the preparation of pharmaceuticals is of a higher grade of purity than ordinary industrial casein. While the requirements may vary in individual cases, casein used in medicinals should have an ash content not greater than 2.0 per cent, and a fat content not over 1.0 per cent.

In the manufacture of this high-grade casein special precautions must be taken. The casein should be precipitated under conditions which preclude contamination with other compounds present in milk, except those that are brought down by occlusion. An excellent method for producing a pure casein on a large laboratory scale is that of Van Slyke and Baker.[1] The product obtained by this method is remarkably low in ash, and free from inorganic phosphorus, calcium and hydrolytic products. The ash content is about 0.10 per cent, and the phosphorus content is 0.80 per cent. The product is a fine, white powder which dissolves at once in dilute solutions of monobasic alkalies, and also in excess of lime-water, to a clear solution. This grade of casein is much purer than that used in medicinal products. Except in a few isolated cases, a casein of this purity is not really necessary for the manufacture of most medicinal compounds.

A distinct feature of the Van Slyke and Baker method is the precipitation of the casein in a very finely divided form. This method cannot be used for the production of large quantities of casein as most factories lack the necessary equipment. To overcome this difficulty, Clark and his co-workers [2] have developed a method for producing a high-grade casein, which can be put into large scale operation. This method, which is known as the "grain-curd" process, will be found fully described in Chapter 3. It produces a casein which is much purer than the ordinary industrial variety and which, for most purposes, is well adapted for the manufacture of medicinal products.

To eliminate confusion, the term "casein" used in describing the properties and method of manufacture of medicinal products in subsequent pages is restricted to the free, uncombined protein, the amphoteric electrolyte whose iso-electric point is pH 4.6. The substance normally

present in milk contains calcium and this is known as "neutral calcium caseinate."

1. Casein in Medicinal Foods

Milk is the best food for the young and growing organism, and since casein is the most abundant and characteristic protein in milk, it is natural that it should form an important constituent of many patented and medicinal foods. As a protein, it belongs to the class of phosphoproteins. In its purest form it contains not less than 15 per cent of nitrogen, is rich in amino-acids, and also contains about 0.8 per cent of phosphorus and 0.8 per cent of sulfur. Casein is a protein which is easily and fully converted into peptone and other more simple derivatives, and, therefore, is readily absorbed and assimilated. Flesh preparations are also rich in amino-acids and are easily assimilated, but they do not compare with casein in regard to the amount of organic phosphorus.

The function of casein in medicinal foods is to supply special needs in the nutrition of the sick, convalescent, infants, and in the dieto-therapy of diabetes. The true nutritive value of these foods, of course, is based on the usual calorific values of their constituents, namely, fats, proteins, and carbohydrates. Each gram of dry casein yields about 4.1 calories. Uncombined casein is difficultly soluble in water and is not used in this form. It is usually converted to the soluble sodium caseinate or the partially soluble calcium caseinate. The casein used in the manufacture of dry protein foods is relatively free from odor and taste and is generally lighter in color than ordinary industrial casein. The limits for impurities for edible casein are given under Calcium Caseinate N.N.R. and Casein N.N.R.

Calcium Caseinate N.N.R.—This is casein from cow's milk rendered partially soluble by combination with calcium, and containing not less than 1.4 per cent nor more than 2.1 per cent of calcium. Calcium caseinate is made by dissolving a low-ash casein in lime water of sufficient strength so that the turbid suspension resulting is neutral or faintly alkaline to phenolphthalein. The suspension is carefully dried and then powdered. Calcium caseinate is a yellowish-white, odorless powder, having a slightly saline taste. Its solution is neutral or very faintly alkaline to phenolphthalein. The New and Non-Official Remedies [3] states that calcium caseinate should contain not less than 1 per cent of calcium, 0.6 per cent of phosphorus, and not less than 14 per cent of nitrogen calculated on a moisture free basis. It should contain not more than 10 per cent of moisture and the fat must not exceed 2.5 per cent. Its purity is ascertained according to the methods outlined in Chapter 12.

Calcium caseinate is used to increase the protein content of milk in order to obtain a product which is relatively low in fat and lactose and

high in calcium salts. It may also be added to gruels and other liquid foods. The diarrheal diseases of infancy are often treated with these modified foods. For infants and children the dose is approximately 10 grams of calcium caseinate to 500 cc. of solution; for adults 20 grams to 1000 cc. of skimmed milk or other diluent. Calcium caseinate is marketed under various names, as, Casec, Larosan-Roche, and Protolac.

Casein N.N.R.—This represents the protein separated from milk by the action of acids or enzymes and purified. Casein is soluble in ammonia water and in aqueous solutions of the fixed alkali hydroxides; insoluble in water, alcohol, ether, chloroform, or benzene. A suspension of casein in water slightly reddens blue litmus paper, but casein has both acidic and basic properties. The requirements of the New and Non-Official Remedies [3] for edible casein are as follows:—The ash content must not exceed 2 per cent; the loss at 100° C. must not be more than 10 per cent; the fat must not exceed 1 per cent; and the nitrogen must correspond to not less than 15 per cent of the moisture-free material.

This brand of casein is mixed with other ingredients of the diet in order to increase the protein content. A diet of this kind is desirable for feeding infants, adults, convalescents, under-nourished persons, and also in diabetes where foods poor in carbohydrates and fats are indicated. The quantities used vary in accordance with the needs of the individual and the purpose of the feeding. In severe cases of diabetes a high protein diet must be avoided, as it has long been known that sugar can be formed from protein.

Diaprotein Prepared Casein Flour N.N.R. is a commercial diabetic food, consisting of casein to which has been added 4 per cent of a leavening mixture composed of sodium bicarbonate, monocalcium phosphate, and sodium aluminium sulfate. This is sold in the form of a flour which may be used in the preparation of bread, cake, or muffins.

Caseinhydrol, another proprietary product, consists of casein, calcium phosphate, and magnesium oxide. It is also used in the dieto-therapy of diabetes.

Sodium caseinate.—This salt of casein is marketed under the trade name of Nutrose.[4] It is a white, inodorous, and tasteless powder, containing about 65 per cent protein, slightly soluble in cold, and very soluble in warm water. It is prepared by dissolving moist casein in a solution of sodium hydroxide, evaporating the solution under vacuum, and powdering the residue after washing successively with alcohol and ether. Nutrose is given in milk or water in large doses as a non-irritant nutrient, in the treatment of convalescent, under-nourished, or anemic patients.

According to the Casein Manufacturing Co. of New York City, their "Nucleo," another sodium caseinate, is made as follows:—"The curd of fresh skim-milk is precipitated with just sufficient hydrochloric acid to

secure a clear separation, using the usual well-known method. The curd is then washed repeatedly and redissolved with a minimum amount of bicarbonate of soda. It is then precipitated again with hydrochloric acid, thoroughly washed with several wash waters, and again dissolved with a minimum amount of bicarbonate of soda. The product is then diluted to about the consistency of cow's milk, so far as solid content is concerned, and dried on a double roller dry milk machine." The approximate analysis given for this product is as follows:

Protein (N x 6.25)	81.31	Per cent
Fat	0.27	" "
Nitrogen	13.01	" "
Ash	5.10	" "
Moisture	5.20	" "
Sugar	none	
Alkalinity (as Na_2O)	0.041	" "

Ammonium Caseinate.[4]—This product is made by passing ammonia fumes over dry, finely powdered casein, until a trial shows that the compound is almost completely soluble in water. It is marketed under the trade name of Eucasein, and is used as a nutrient for consumptives and patients suffering from stomach and lung trouble. The dose is one tablespoonful 2 to 4 times a day in hot coffee, chocolate, or soup. Thirty to forty grams of Eucasein are equivalent to 24 to 32 grams of egg albumin as an energy yielding food.

Proprietary Preparations.—There are many other proprietary and patented food preparations which contain casein in amounts from 20 to 95 per cent. Among these may be mentioned:—*Sanatogen*, which contains about 90 per cent of casein and about 5 per cent of sodium glycerophosphate. Sanatogen is made by triturating the casein with the sodium glycerophosphate and then adding about 0.5 per cent of sodium bicarbonate and 0.5 per cent of sodium chloride. The mixture is dried at a low temperature, ground and sieved. It is recommended as a food and a tonic.

Plasmon [4] is made by mixing casein with a sufficient amount of sodium carbonate to render the casein soluble. The mixture is heated to 70° C. in a mixing tank and then CO_2 is passed through the mass. The product is disintegrated into small pieces which are spread over trays and dried quickly. Plasmon is sold as a light yellow powder, which is completely soluble in hot water. In insufficient water it swells markedly and may even form a gel. The powder possesses a slight, sweet, milky taste. It finds use as a food and is usually mixed with soups, coffee, tea, etc., in order to utilize its nutritive and easily digested properties.

Sanose is a casein preparation containing about 80 per cent of casein and 20 per cent albumoses. It is a white, odorless, tasteless powder,

which forms a milky suspension in water. It also is used as a food and a tonic.

Caseinphosphorol [4] is a compound of casein very closely related to Sanatogen, and is used similarly.

Eulactol, Higgins' Casein Food, and Guttman's Nutrient Milk Flour are other medicinal foods which are designed to meet the special needs of the sick.

Some of the proprietary foods have earned a place in dietetics. Some, however, have been sold at high prices under extravagant claims, and the prices charged have been entirely out of proportion to the true food value which they contain.

Determination of Casein in Medicinal Foods.—The casein in patented and proprietary food products may be determined as follows: [5] Weigh accurately about 1 gram of the substance, dissolve in dilute potassium hydroxide solution, precipitate the casein with dilute acetic acid, collect the precipitate and determine its nitrogen content by the usual Kjeldahl method. The nitrogen obtained expressed in grams, multiplied by the factor 6.38 gives the weight of casein in the sample taken. If the substance contains other proteins besides casein the procedure is modified as follows:—The total nitrogen in the product is determined by the Kjeldahl method. Subtract from this figure the casein nitrogen and the difference equals the nitrogen in the form of other proteins.

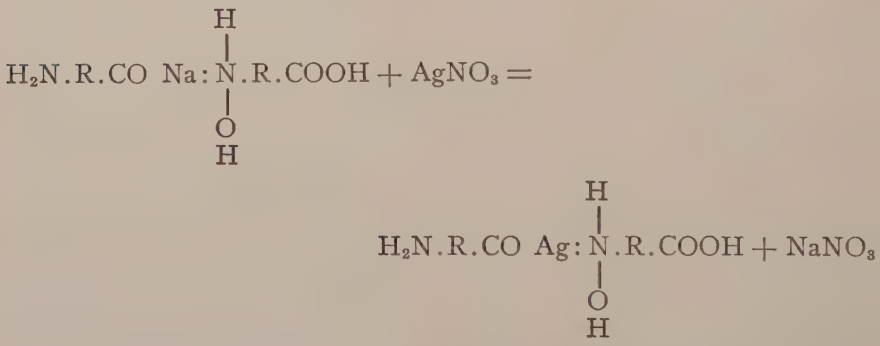
2. Compounds of Casein with Heavy Metals

As casein is amphoteric, it forms salts with either bases or acids. A suspension of uncombined casein in distilled water is acid to litmus, and also acts as an acid in reacting with carbonates and bicarbonates, forming a salt with the base. According to Osborne [6] the sodium and potassium, as well as the alkaline earth caseinates may be made in this manner. Although casein behaves as an acid and is usually considered as such, Bancroft [7] ascribes this behavior to a marked adsorption of the base. Robertson [8] states that casein forms multiple salts with potassium hydroxide. These salts contain from 11.4×10^{-5} to 182.4×10^{-5} equivalents of the base per gram of casein. Zoller,[9, 10] however, has proved that at 180×10^{-5} equivalents of base per gram of casein, the reaction of the solution is so alkaline that not only ammonia but also sulfur and phosphorus are broken loose from the casein molecule. It is incorrect and confusing to state that the true casein molecule can combine with more than 90×10^{-5} equivalents of base without taking into consideration the decomposition of the casein molecule into simpler derivatives. Zoller's observation is a very important one, as many investigators have reported heavy metal salts of casein as definite caseinates containing a definite con-

tent of heavy metal, when in reality they were dealing with mixtures of salts of heavy metal and casein hydrolytic products.

(A) Compounds of Casein with Silver

Rohman and Hirschstein [11] prepared compounds of casein with silver nitrate as follows:—A two to four per cent solution of sodium caseinate, neutral to phenolphthalein, was added to an excess of silver nitrate solution; the resulting precipitate was collected on the filter and washed with distilled water until the filtrate tested with diphenylamin gave no test for nitrates. The dried precipitate contained 85×10^{-5} equivalents of silver per gram of substance. At neutrality to phenolphthalein casein neutralizes 80×10^{-5} equivalents of NaOH. The salt formed, therefore, is the result of interaction between one molecule of silver nitrate and one molecule of caseinate according to the equation: [8]



This compound is undoubtedly a true silver caseinate containing about 9 per cent of silver. It is only slightly soluble in water (0.47 per cent) and is, therefore, of little value in therapeutics.

Silver compounds of casein are used in medicine as substitutes for silver nitrate. The latter is highly efficient as an antiseptic but its practical use is limited on account of undesirable side reactions, namely:—irritation, pain, astringency, and corrosion. It has been shown that the high antiseptic and irritant action of silver nitrate is essentially due to the free silver ions. Consequently, if the silver ions are reduced, the antiseptic and irritant action is lessened. This principle is utilized in the preparation of protein-silver compounds whereby products are obtained which combine a fair degree of antiseptic action with a much smaller degree, or even absence, of irritant action. This is accomplished by changing a very large part of the active silver ions into inert forms of silver, such as colloidal metallic silver, silver oxide, or indefinite compounds

of silver with protein split products. In other words, the antiseptic action of colloidal protein-silver preparations is due to the presence of low concentrations of silver ions, a concentration so low as to be practically non-irritating, but sufficient to show distinct antiseptic activity when tested against certain micro-organisms.

Colloidal protein-silver compounds have been found to be very efficient for the prophylaxis against gonorrheal infection. Gonococci are killed in a few minutes on exposure to a 0.25 to 1.0 per cent protein-silver solution. Pilcher and Sollman[12] have developed an excellent method for measuring the free silver ions in protein-silver preparations and allied products. This method, which depends on the fact that silver ions restrain the growth of yeast cells, and hence the formation of gas, has been included in the U. S. Pharmacopœia, X.[14] A determination of the degree of restraint in watery solution enabled them to group the commercial protein-silver preparations in four classes:[13]

(a) Protargin Strong Type,
(b) Protargin Mild Type,
(c) Collargol Type,
(d) Electric Type.

(a) Protargin Strong Type.—Protargin strong compounds contain the lowest percentage of silver, not less than 7.5 nor more than 8.5 per cent of silver (Ag), but have the strongest germicidal action, and are distinctly irritant. They are intermediate in their therapeutic action between silver nitrate and protargin mild. The following products listed in the N.N.R. belong to this group: proganol, protargentum and protargol. These commercial preparations are made by dissolving reduced silver oxide in an excess of either albumoses or gelatin. They will, therefore, not be considered further.

(b) Protargin Mild Type.—Protargin mild compounds contain from 19 to 25 per cent of silver (Ag), but are quite non-irritant, due to the fact that they contain when in solution less silver ions. The following products are listed in the N.N.R.: argyn, argyrol, cargentos, silvol, solargentum and vargol. Of these cargentos is the only one which is made by dissolving silver oxide in a casein solution. The others are made by using some other protein. Cargentos will be described in detail below.

The Collargol and Electric types of silver-protein compounds should not be considered here, as they do not contain casein as a form of protein.

Cargentos.—This is a casein-silver compound containing 20 to 25 per cent of metallic silver and 50 to 60 per cent of protein in the form of casein-split products. It is made by adding moist silver oxide to a partially hydrolyzed, low-ash casein, the hydrolysis being brought about by means of an alkali. The colloidal suspension is heated until no precipitate

is obtained on the addition of a solution of sodium chloride to a few cubic centimeters of the casein-silver salt. It is then evaporated to dryness in an air oven. The product is marketed in the form of black, non-hygroscopic scales having a metallic luster. It mixes readily with water, forming a clear, colloidal suspension. When tested according to the method of Pilcher and Sollman (U. S. P. X.) it falls into the second class, or the Protargin Mild Type. (See Table I, p. 234.) Cargentos also meets the other requirements of the U. S. P. X. The addition of 2 cc. of an aqueous solution of sodium chloride (1 in 100) to 10 cc. of an aqueous solution of the salt (1 in 100) causes no turbidity. This shows the absence of any appreciable ionizable silver. Ferric chloride T.S. added to its aqueous solution (1 in 100) discharges the dark color and a precipitate is gradually produced. A few drops of Cargentos solution (1 to 10) when placed in the nostril produces no sensation of irritation. It differs in this respect from the Protargin Strong Type.

Cargentos is used therapeutically on mucous membranes of the body for its antiseptic and germicidal value. It has been used with satisfactory results in the treatment of inflammation of the mucous membranes of the ear, eye, nose, throat, rectum, etc., applied every two to four hours, in a 5 to 50 per cent concentration.

Argonin.—This is a compound of casein and silver containing about 10 per cent of metallic silver. It approaches in composition a true silver caseinate and consequently is difficultly soluble in cold water. Argonin is a fine white powder forming a coarse suspension in warm water. When ready to use, a 1 to 3 per cent suspension is made and passed through gauze or other similar filter. The fine suspension passing through the filter is used therapeutically in the treatment of gonorrhea and eye infections.

(B) Compounds of Casein with Mercury.—Attempts have been made to produce casein-mercury compounds which, while therapeutically active, would be relatively less corrosive, less irritating on injection, and less toxic than soluble inorganic salts. Mercury caseinate is made by precipitating a solution of neutral sodium caseinate and mercuric chloride with alcohol, or by evaporating the mixture. It is used as a substitute for other mercury preparations.

Another mercury-casein compound for use in therapeutics has been made by allowing a neutral solution of sodium or potassium caseinate to react with mercuric iodide solution (G. P. 288,965). The final product, containing 15 to 20 per cent of mercuric iodide, is supposed to be suitable for medicinal use.

These preparations have not been extensively used in therapeutics, but have been largely abandoned in favor of mercuric succinimide and organic mercury compounds.

(C) Compounds of Iron and Casein.—Iron-casein compounds have been developed to eliminate the astringent taste and local irritant action of simple iron solutions. These complex iron preparations do not give the ordinary tests for ferrous or ferric ions because the iron in them is either masked or in combination with the protein radicle and is, therefore, nonionic. There is no evidence that the compounds are definite chemical salts; in all probability they are indefinite mixtures of iron oxide and iron caseinate. They are not decomposed by dilute acids or by gastric digestion, and are slowly soluble in dilute alkalies. The principal use of iron-casein compounds is in the treatment of anemia and chlorosis.

Ferric Caseinate,[4] also called ferric nucleo-albuminate, is prepared by adding a freshly prepared solution of ferrous lactate to calcium caseinate. It is an odorless and tasteless powder, containing 2.5 per cent of iron; it is insoluble in water, but soluble in weak alkalies and ammonia. This iron preparation is recommended as a tonic and is said to be easily absorbed.

Proferrin N.N.R., iron nucleo-protein, is a compound of iron and casein made by heating an alkaline solution of casein with an iron salt. The mixture is then treated with acetic acid until precipitation is complete. The precipitate is completely dried, ground, and sieved. Proferrin contains about 10 per cent of iron and 0.5 per cent of phosphorus, the latter being naturally present in casein. It is a brown powder, almost odorless, tasteless, insoluble in water and dilute acids, but slowly soluble in alkalies. Proferrin is not affected by the gastric juice, does not precipitate albumin, and is free from astringent action on the stomach. It is readily decomposed in the intestine and is rapidly absorbed. This preparation is recommended wherever blood impoverishment exists, either as deficiency of hemoglobin or diminution of red blood corpuscles.

Triferrin [4] is another proprietary iron-casein compound made by digesting casein with pepsin, and precipitating the soluble casein derivatives with a ferric salt. It is a red brown powder, insoluble in water, but soluble in weak alkalies. Triferrin contains 16 to 22 per cent of iron, 9 per cent of nitrogen, and 2.5 per cent of phosphorus. Therapeutically its use is similar to other masked organic iron preparations.

(D) Aluminium Caseinate [4] is a yellow-white, tasteless powder, containing about 5 per cent of aluminium; it is insoluble in water but sparingly soluble in saliva and quite soluble in dilute acids. This compound has been used in medicine as a remedy for intestinal catarrh, the aluminium acting as an astringent and an antiseptic. It has gradually fallen into disuse, being replaced by bismuth salts.

(E) Compounds of Bismuth and Casein.—A number of compounds containing bismuth oxide have been prepared with albumin or casein as a base. In general, they are insoluble and have a low antiseptic

power and a slight germicidal activity. Their action is a mechanical one as they protect inflamed or irritated surfaces. When dusted on wounds, they form a crust beneath which healing takes place. This is brought about by the very fine subdivision of the bismuth compound which possesses marked drying properties.

Bismuth Formic Iodide is a well-known bismuth-casein compound. It is made by adding a solution of potassium iodide to a solution of casein and treating the mixture with a bismuth salt, the resulting precipitate being washed and then allowed to react with formaldehyde. The precipitate is dried, ground and sieved. It is a yellowish powder, insoluble in water, containing about 1.4 per cent of iodine organically combined. It forms the basis of a dusting powder used extensively as an effective astringent, alterative, and healing dressing for wounds, ulcerations, and skin diseases. The iodine exerts a slight but effective antiseptic action and in so doing stimulates phagocytosis and diminishes the secretion of the wounds. The bismuth gives a mechanical protective action and thus contributes to the drying property of the powder.

3. Casein Addition Compounds

Solutions of casein react readily with halogens, tannic acid, and formaldehyde, to form substitution and addition compounds.

(A) Casein-Iodine.—With iodine casein forms compounds which are extensively used in medicine. Preparations of this sort appear to be less irritating to the digestive tract and do not show the sharp, disagreeable symptoms of iodine, as do the metallic iodides. As the iodine is organically bound this type of compound is not germicidal. When given orally, these compounds are absorbed and may increase the amount of thyreoprotein and influence metabolism.

Iodo-casein N.N.R. is a commercial preparation made by treating a solution of sodium caseinate with iodine and potassium iodide. Acetic acid is added until precipitation is complete, and the precipitate is washed free of iodine, collected and thoroughly dried. It contains about 18 per cent of iodine and is a brownish yellow powder, practically odorless and tasteless, insoluble in water, and slightly soluble in dilute acid solutions. It is completely dissolved and digested by the alkaline intestinal secretions, being absorbed in the form of soluble iodides. Iodo-casein is recommended as a substitute for inorganic iodides in the prevention and treatment of simple or endemic goiter.

(B) Casein-Formaldehyde.—Solutions of caseinates react with formaldehyde to form insoluble compounds. The formaldehyde condenses with the $-NH_2$ groups in the casein molecule, forming methylene linkages with the elimination of water. The basicity of the casein molecule

is reduced and the acid character of the carboxyl radicle made more pronounced.

Proteol [15, 4] is a condensation product of casein and formaldehyde. It is an insoluble, tasteless, yellow powder, used as an antiseptic in the treatment of wounds. This compound may also be prepared in the form of a thick paste and used on cuts and bruises as an elastic coating to prevent bacterial infection.

(C) Casein-Tannic Acid Compounds.—Casein, like gelatin and albumin, precipitates tannin from solution; in fact, Nierenstein [16] proposed the use of casein in place of gelatin for the determination of tannic acid. When taken internally, tannic acid in uncombined form dissolves rapidly in the stomach and produces disagreeable reactions such as irritation, nausea or vomiting. To overcome this, many protein-tannin compounds have been introduced. The medicinal value of these preparations is based on the fact that protein-tannin combinations are only sparingly soluble in water, partially soluble in dilute hydrochloric acid, but almost completely soluble in dilute sodium bicarbonate. Therefore, when taken internally, they should show little action in the stomach but much greater action in the intestines. This ideal behavior has not been entirely obtained in test tube experiments. The casein-tannic acid preparations used in medicine are intended primarily as intestinal astringents in the treatment of diarrhœa.

Little is known about the chemistry of the casein-tannic acid combination. As the compounds used in therapeutics are made by precipitating tannic acid with a solution of sodium caseinate, it is very likely that the precipitate formed is of a physical character, a loose "adsorption" compound. The isoelectric point of casein is about pH 4.6. In sodium caseinate the casein molecule is negatively charged and it has been proved that tannins [17] in aqueous solution are colloidal and are also negatively charged. As both the colloidal suspension of casein and that of tannin are negatively charged they cannot combine in the same manner as two oppositely charged ions would do. The precipitate formed, therefore, by adding sodium caseinate to a tannin solution is not the result of colloidal behavior between two oppositely charged particles but appears to be one of a purely physical nature.

Tannocasein has been recommended as a substitute for Tannalbin, Tannigen, and other intestinal astringents. It is made by adding tannic acid and formaldehyde to a solution of sodium caseinate, and treating the mixture with dilute hydrochloric acid. The resulting precipitate is pressed, dried, and ground to a powder. It resists gastric digestion but is dissolved in the intestinal tract.

Protan N.N.R. is another widely used casein-tannin compound, made by adding a solution of tannic acid to an alkaline solution of casein.

The resulting precipitate is separated, dried, and marketed in the form of a light brown powder. Protan contains approximately 50 per cent of tannin and about 45 per cent of casein. When tested according to the method outlined in the N.N.R.[3] it is not more than 10 per cent soluble in water; not more than 25 per cent soluble in dilute hydrochloric acid (0.08 N), and about 80 per cent soluble in 1 per cent sodium bicarbonate solution. When placed on the tongue and chewed for two minutes it is no more astringent than 0.1 gram of a mixture made from 20 grams of corn starch and 0.1 gram of tannic acid. Protan has an astringent action throughout the entire intestinal tract and is recommended in all forms of diarrhœa.

4. Medicinal Casein Emulsions

Both acid and alkaline solutions of casein hydrate well and form colloidal solutions which yield excellent emulsions. The protective action of casein as judged from its gold number is almost equivalent to that of gelatin. It is certainly superior to gum acacia which is used extensively in official pharmaceutical emulsions. Within certain limits, that is, barring external decomposition, the emulsifying power of casein follows closely its solubility. As casein passes through its isoelectric point, pH 4.6, it becomes almost insoluble, is least ionized, and its protective action approaches zero, but as its solubility increases on the acid side of the isoelectric point the protective power increases accordingly.

Casein solutions yield emulsions of the oil-in-water type, emulsions in which the external or continuous phase is water and the dispersed phase is an oil. In this respect casein behaves similar to gelatin, albumin, saponin, sodium oleate, and other alkali soaps, but different from gum dammar, lanoline, calcium oleate, and other heavy metal soaps which yield emulsions of the water-in-oil type.

Casein as an emulsifier has been used in pharmacy in a rather limited way in spite of the fact that such emulsions are more stable and perfect [18] and are supposed to be more readily retained by the stomach, than acacia emulsions. The probable reason for this is, that casein in solution is easily decomposed; in fact, it is a culture medium for bacterial organisms, and, unless the preparation contained an antiseptic preservative, it would undergo bacterial decomposition in a very short time.

Saccharated Casein.[18]—This has been introduced in pharmacy in the form of a white powder. It "is prepared by heating one gallon of cow's milk to 104° F., adding two fluid ounces of water of ammonia, allowing the whole to stand a day and separating the lower milky liquid from the oily liquid on top. The milky liquid (lacto-serum) is treated with acetic acid until the casein is precipitated. After washing the precipitate thoroughly with water at 104° F. it is collected on a muslin strainer and

pressed; a weighed portion of the casein is dried and the percentage of moisture ascertained; the damp cake of casein is then triturated with three and a half ounces of powdered sugar and eight parts of sodium bicarbonate for every one hundred parts of casein (dry). Prolonged trituration and the addition of more powdered sugar, until it amounts to nine parts in one hundred, result in the formation of a paste, which must now be dried by a gentle heat not above 86° to 90° F. After complete drying it is powdered and sifted. To make a casein emulsion of a fixed oil fifteen parts of the oil are gradually incorporated with a mucilage previously made with fifteen parts of saccharated casein and five parts of water. When a perfect emulsion is formed the other ingredients are added."

Casein Ointment.[19]—This is made by dissolving 34.5 parts of potassium hydroxide and 8.5 parts of sodium hydroxide in 5000 parts of water and then dissolving in this 1400 parts of casein. Seven hundred parts of glycerine and 50 parts of phenol are added to the casein solution and when dissolved 2100 parts of white petrolatum and 50 parts of zinc oxide are then incorporated. The whole is made up with water to make 10,000 parts. The ointment has the consistency of a thick cream and is incompatible with acids and salts.

Certain forms of casein creams are known as "rolling creams" because when rubbed over the surface of the skin they take the dirt out of the pores by rolling up in small particles which can be brushed off. They are extensively used as cosmetics and usually form the basis of non-greasy skin creams. They contain zinc oxide and gum tragacanth or other suitable medicaments.

Recently the emulsifying and protective power of casein has been utilized in the development of antiseptic and mildly germicidal silver-emulsion preparations which are much superior to the ointments generally used. The bases of ointments are only indirectly or mechanically antiseptic in that they exclude air, dust and organisms from the covered area. Their antiseptic or germicidal value depends entirely on the drug which is incorporated in them.[15] Ointments are in reality emulsions of the water-in-oil type, the external phase being an oil, vaseline or lanoline. The dispersed phase is water in which is dissolved the active germicidal ingredient. Therefore, ointments are inefficient vehicles because the oily base, and not the antiseptic, comes in contact with the surface of the wound. The antiseptic or germicidal power of the drug is not available to act on the bacteria present and destroy them. Hence the uselessness of many antiseptic ointments.

By using casein as an emulsifier the oil-in-water types of emulsion result. The antiseptic is dissolved in the water which becomes the external phase, while the inert base forms the dispersed phase. The antiseptic

comes in contact with the surface of the wound and destroys the organisms present. The superiority of this type of emulsion or ointment is evident.

Cargel is a heavy-brown silver emulsion of the oil-in-water type, the emulsifier being a mixture of alkaline and alkaline earth caseinates. The base is lanoline which tends to produce an emulsion of the opposite type, but as casein is more strongly hydrated, the oil-in-water type prevails. The active antiseptic is 1 per cent metallic silver added to it in the form of a colloidal silver solution, and as the external phase is water, the silver can readily act on bacteria and destroy them. Standard suspensions of bacteria, immersed in Cargel, are killed in three to five minutes. It inhibits the growth of yeast to the same degree as a 5 per cent solution of a Protargin Mild silver salt. Unlike ointments of the water-in-oil type, the emulsion mixes readily with water and can easily be washed off from the applied area. The emollient properties of the preparation make the action of the silver comparatively non-irritating so that it does not interfere with cellular activity or normal secretion. On application to a wound or other surface, Cargel forms a coating which is smooth and dry. Its antiseptic action is manifested directly by the action of the silver and indirectly by the formation of a casein coating which mechanically excludes air, dust, and organisms. It is useful in the treatment of wounds, abrasions, and burns, where infection must be checked and the surface protected.

Arkase is also a silver-casein emulsion preparation resembling Cargel in its action as a local antiseptic. It has been used quite extensively in skin eruptions and also in the treatment of nose and throat infections. Its germicidal value is equal to that of a 1 to 75 phenol solution. Arkase is claimed to possess hitherto unattainable penetration of skin and mucous membranes.

TABLE I

PILCHER-SOLLMAN YEAST TEST SHOWING THE INHIBITING ACTION OF FREE SILVER IONS IN CERTAIN PREPARATIONS ON THE GROWTH OF YEAST.*

Preparation	Amount Placed in Fermentation Tubes in cc. and Gas Formed in Each Expressed as Per cent.				
	0.4	0.6	0.8	1.0	1.5
Cargel: oil-in-water emulsion containing 1% Ag.	50	bubble	none	none	none
Water-in-oil emulsion containing 1% Ag.	100	100	100	100	100
Cargentos: protargin mild type containing 1% Ag.	70	50	25	none	none
Silver nitrate 1 in 2000	50	35	5	bubble	none

* Unpublished resulted obtained in writer's laboratory.

In general, to summarize, casein is of value not only as a food and as an emulsifying agent, but it can also be used as a substitute for albumin wherever an albuminate or a proteinate of a heavy metal is desired. Like albumin, the function of casein is not so much to form a definite chemical entity with corresponding new chemical properties, but rather to act as a vehicle for the heavy metal or other active principle. The casein may form adsorption compounds or may act as a protective colloid and thus "mask" or modify some of the undesirable properties of the chemical, such as irritation, astringency, corrosion, or pain on injection.

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Chapter 9

Casein in Foods

By H. A. Schuette

Our newer knowledge of nutrition has been able to explain in some degree the conspicuous role which milk and milk products have played in the human dietary from time immemorial. Not only has milk been long recognized as a valuable food, but the nutritive value and ready assimilability of its various components have also been appreciated, and large quantities of milk which find no immediate use as a liquid food have always been preserved and used in the form of cheese and butter.

In the uncombined state in which commercial casein is marketed as a by-product of the creamery, it is not a food product. In its modified condition as cheese, however, it has always had a place in the dietary of all nations, although its value when measured in terms of present-day nutrition yardsticks may not have been fully appreciated. Commercial casein suitable for the needs of the arts does not possess the requisite degree of fineness, purity, solubility, and digestibility for alimentary purposes, where a product substantially free from color and odor and of a fine texture is necessary.

From the standpoint of nutritive value, casein compares very favorably with the proteins of meat.[1] In the form of its amino acids, it is readily absorbed in the intestinal canal, metabolism experiments having shown a utilization[2] of about 98.5 per cent. This value is in excess of that obtained with proteins of vegetable origin. Osborne and Mendel,[3] in a study of the biological values of individual proteins when fed at an 18 per cent level as compared with other substances in the diet, demonstrated that casein is superior to all other proteins in promoting growth, and Mitchell[4] found it to be more effective than egg white in increasing body weight.

Casein is described as "complete" because it is composed of amino acids which are adequate for growth and nourishment, being satisfactory in kind, quality, and quantity. Among its hydrolysis products are numbered some fifteen amino acids, significant among them being tryptophane and glutamic acid. Others are alanine, valine, leucine, phenylalanine, tyrosine, serine, cystine, proline, hydroxyproline, aspartic acid, arginine,

lysine, and histidine. Unanimity of opinion as to the presence of glycine apparently does not exist, although the weight of experimental evidence points to its absence.

Besides its amino acids, casein also contains phosphorus and is therefore classified as a phosphoprotein. The significance of this element in the growth, development, and functional activities of the animal organism has received but tardy recognition, yet it is pertinent to note that it has been demonstrated that fully 90 per cent [5] of the phosphorus of casein is utilized by the body.

The foregoing considerations, it is believed, definitely establish the food value of casein and at least partially explain why casein has been utilized in such a variety of ways for alimentary preparations.

Casein is also prized for its contribution of vitamins to the diet. Animal experimentation has demonstrated, however, that casein precipitated from a solution of its sodium compound and then shaken with Fuller's earth in the presence of hydrogen peroxide becomes inadequate in nutritive efficiency.[6] Such a casein is claimed to be vitamin-free.[7]

In combination with alkalies, as in the so-called caseinates, casein is soluble in water, and is therefore readily acted upon by the digestive juices. The caseinates have a satisfactory degree of stability if kept dry, and in this condition are without odor. Experimental evidence shows that from a nutritive standpoint their value is very similar to that of casein itself.[8, 9]

Not only is casein acceptable from a nutritional standpoint, but as a typical colloid its physical properties have been taken advantage of in the preparation of the many food products in which it plays a conspicuous part. Casein-containing preparations are numerous, for "within recent years," writes Seaber,[10] "there has been a growing demand for food substances which shall take the place of the flesh-building part of meat. This is perhaps largely owing to the increase of vegetarianism and though many hardened meat eaters will look with disgust upon such preparations, yet they are very valuable on account of the nourishment they are capable of affording without overloading the system."

The use of casein or its compounds in foods finds expression in instances of which the following are typical: reconstituted or synthetic foods; foods for children, for the diabetic, and the dyspeptic; in infants' foods; in reconstructed, modified, malted, and "albuminous" milks; in food adulterants; bakers' wares; as ingredients of so-called egg substitutes; as ice-cream filler; in soup tablets; as an adjunct in the manufacture of oleomargarin; and in milk chocolate and cocoa.

Reconstituted or Synthetic Foods

The knowledge that a liquid closely resembling milk and cream can be made by emulsifying unsalted butter fat in a solution or suspension of skim-milk powder is not new. The ice cream industry uses this procedure very widely.

From an economic standpoint and in the interests of food conservation, desiccation of milk may sometime be desirable, if not necessary, and from purely monetary considerations storage and transportation of the community's milk supply on a water-free basis offer attractive possibilities. The ethical aspects of handling reconstructed milk under its own label and with complete information as to source and method of preparation are beside the purposes of this discussion. Skim-milk powder containing casein, sweet or unsalted butter as a source of butter fat, and water, form the ingredients which when properly mixed result in a product for which names such as "remade," "emulsified," "reconstructed," "recombined," and "rehydrated" have been suggested. The exigencies of the late war brought such a product into prominence at Nitro, W. Va., under conditions which made possible its study from a commercial standpoint.[11]

A stable suspension of casein in water for use as a milk substitute in bread baking was made by Dunham.[12] He dissolved casein in an alkali, made the solution acid to litmus by the addition of an acid citrate, thus precipitating the casein in a colloidal form, and then dehydrated the mass in a milk-drying machine of appropriate design. A somewhat similar use of a citrate is claimed by Backhaus [13] who converted casein into an "appetizing food product" by the use of trisodium citrate in the presence of sodium bicarbonate or trisodium phosphate.

From German sources comes information [14] of a casein product which may be boiled with water without the separation of a gelatinous precipitate. This is made by mixing casein or its salts with albumin or peptone-containing substances. Monhaupt's patented preparation, referred to later in this chapter, also belongs in this class.

It may be of more than passing interest to note that König,[15] some twenty-five years ago, took the position that no pecuniary advantage accrues to agriculture in the utilization of skim-milk for the production of patented preparations of the type exemplified by Plasmon, "Kalk-casein," Nutrose, Sanatogen, Eukasin, Galaktagen, Eulaktol, "Milcheiweiss 'Nickol'," and "Sanitätseiweiss 'Nickol'," in view of the fact that such proteins cost more than those of milk and cheese.

Foods for Children

A milk food known as Guttmann's nutrient flour, whose chief characteristic is a high content of readily assimilable carbohydrates, appeared

on the German markets some years ago. This is said to be very digestible and especially adapted to the needs of children and of dyspeptics. According to Jolles [16] its base is skim-milk casein to which are added prepared carbohydrates, such as gluten-free oatmeal, and a small quantity of aromatic substances for the purpose of imparting a flavor to the mixture. Carbohydrates made up about 65 per cent of the dry matter, nitrogenous matter is present to the extent of 20 per cent, while the fat content is 4 per cent.

Sell [17] prepares from casein what is designated as an easily digestible food product. At the will of the operator its nutritive value may be enhanced by the addition of lactose or its content of solids increased by the addition of a concentrated whey. The action of added kefir "grains" aids in the breaking down of the whole mass. The acidity of the reaction product is reduced, if not entirely neutralized, and then the mass is absorbed on zwieback or similar dry food, and then dried.

For the preparation of a product with some of the characteristics of a baked food, Liebrecht [18] makes an intimate mixture of casein or its salts with animal or vegetable fats and then heats it to 150° to 200° C.

Foods for Diabetics

It is probably not too much to say that there is no universal food for diabetics, for most foods may be suitable under certain conditions for the use of persons afflicted with the disease. The discovery of newer methods of diabetes therapy has rendered obsolete the definition that a food for diabetics contains not more than half as much glycogenic carbohydrates as a normal food of the same class.[19] Although the number of proprietary foods on the market making claim to this doubtful distinction is almost legion, they are being replaced by more satisfactory dietary regimens which, thanks to the discovery of insulin, are based on the carbohydrate tolerance of the patient.

In a compilation [20] of some 630 foods for diabetics, recently made at the Connecticut Agricultural Experiment Station, only five were found in which casein was either known to be present or mentioned on the label. Three of the five samples in question were casein flours whose protein content ranged from 72 to 79 per cent. The analyses of these preparations are given in the table on page 240.

A milk for diabetics said to be sugar-free, of minimum protein but of high fat content, and possessing a pleasant taste, is described in the Milch Zeitung.[21] Information on its practical value is not available, but it is believed that its sale can be only local. Its composition for a "five per cent" product was found to be: fat 4.96 per cent, protein 1.14 per cent (62.5 per cent of which was casein), mineral matter 0.17 per cent,

Composition of Foods for Diabetics

Name	Water	Ash	Nitrogen	Nx6.25	Crude Fiber	Starch	Other Nitrogen-free Extract	Fat
Alpha Best Diabetic Wafer ...	6.81	6.23	7.74	48.38	0.17	...	. 4.89	33.52
Diabetic Casein Flour	11.93	9.16	11.56	72.25	0.14	...	5.73	0.79
Prepared Casein Diabetic Flour.	11.53	9.44	10.93	68.31	0.05	...	9.72	0.95
Diaprotein Casein Flour	11.72	6.35	12.44	77.75	...	...	2.72	1.46
Snow Flake Diabetic Casein Flour	10.41	6.07	12.67	79.19	...	...	3.18	1.15

nitrogen-free substances 1.24 per cent, and water to make 100 per cent. Similarly a ten per cent milk had an indicated fat content of 9.98 per cent with a protein value of 2.29 per cent.

Infants' Foods

It is through the medium of powdered skim-milk that casein enters into the composition of many proprietary foods intended for infant feeding. Often casein itself is used, being modified to a certain extent by the introduction of substances like the phosphates or precipitated in a way to render it substantially ash-free. In an attempt to produce a product which shall possess at least some of the properties of the casein of mothers' milk and be easily digested in the infantile stomach, casein may also be subjected to the action of digestive ferments before it is compounded into infant foods.

Bosworth and Crysler [22] treat milk with disodium phosphate or other substance containing the phosphate radical under conditions which will not disturb the solution of the casein in its natural condition. The milk is then rendered slightly alkaline in order to precipitate insoluble calcium phosphate which is then removed. Neutralization, if necessary, follows this operation. The product so obtained is claimed to be suited for the preparation of milk for feeding infants.

An albuminous milk which Neukirch [23] considers an anti-fermentative and a satisfactory substitute for human milk is prepared by diluting buttermilk with an equal volume of water and two volumes of milk to which casein has been added. The carbohydrate content of the whole is increased by the addition of a dextrin-maltose mixture. The method of preparation followed in the manufacture of Riegel's [24] milk albumin is

of interest because the precipitant used is said to yield an almost ash-free casein which is thrown out of solution in a nonmucilaginous condition. Ethylsulfuric, rather than acetic or sulfuric acid, is used to precipitate the curd from a milk previously diluted.

Nature intended the infant as well as the calf to obtain its nourishment from milk, but while cow's milk is a perfect food for the calf, it is very imperfectly adapted to the infant. The principal differences are that human digestion is largely intestinal while bovine digestion is stomachic, and that cow's milk in the infant's stomach forms large curds. To overcome this condition the milk must be so treated as to cause the curds to form as minute, soft, flocculent particles.

With this in view, Dunham [25] treats casein with a digestive ferment, such as pancreatin, in order partly to peptonize it and at the same time to render it soluble. The curd obtained by acid precipitation is washed free of acid and milk sugar and then dissolved in sodium bicarbonate solution. In this condition it is subjected to the action of pancreatin for twenty to thirty minutes at an elevated temperature. The action is then arrested by drying the product in thin films on cylinders heated to 100° C. After it has been pulverized it is ready for use.

Malted Milk

Malted milk is the product resulting from the combination of whole milk with an extract of malted barley and wheat flour in such a manner as to bring about the full enzymic action of the malt extract. It may also contain sodium chloride, and sodium or potassium bicarbonates. Federal standards require that it contain at least 7.5 per cent milk fat and a maximum of 3.5 per cent of moisture. The whole is reduced to dryness by desiccation. It is the only milk powder made from whole milk that has good keeping qualities and these are ascribed to the protective coating of gluten, sugars, and salts, which surrounds the fat globules.

Horlick's discovery of the method of manufacturing this food product some forty years ago has made available a preparation which combines a high degree of digestibility and nutritive value with the growth-promoting properties of whole milk. Casein enters into its composition as a normal constituent of the milk from which it is made. A typical method of manufacture follows: A mash of wheat flour and barley malt of good diastatic quality is allowed to age long enough to permit complete conversion of the starch into dextrin and maltose, a process similar to that of digestion in the human system. This extract is then combined with whole milk and the mixture reduced to powdered form by vacuum evaporation. The temperature used is sufficient to cause Pasteurization without affecting the digestibility of the product. Hunziker [26] finds that

the total protein content of malted milk varies from 10 to 15 per cent, the casein content lying between 6.4 and 9.25 per cent.

Food Adulterants

It has been reported that some dairymen actually work casein in the form of milk powder or cottage cheese into their butter and cream for the purpose of increasing their water-holding capacity. The practice, however, is rather vigorously opposed within the industry itself.[27] Occasional instances of this form of adulteration of Dutch butter have come to light in the past, the presence of casein making itself apparent in the separation of an unusual amount of coagulated matter from the melted butter. An analysis of such a butter is reported by Racine[28] as: Casein 5.49 per cent, ash 0.77 per cent, water 24.73 per cent, and fat 69.01 per cent.

At the present time, however, food law enforcement officials of the Department of Agriculture report that if the practice exists at all to-day it is strictly local in character and affects only an infinitesimal amount of the production. Since the enactment of the food and drugs act in 1906, particular attention has been given to butter, and adulteration with curd has not been found on any appreciable scale.

Cream is another dairy product which has sometimes fallen into similar evil ways. As thickening agents there have come to light preparations consisting of cane sugar and gum tragacanth, and sometimes casein.[29] Van Slyke[30] reports the fraudulent use of casein and dried and condensed milks for this purpose. The addition of these substances can usually be detected, it is stated, by an unusually high content of solids other than fat. For the detection of casein it is suggested that the suspected cream be diluted with an equal volume of boiling water and that the mixture be strongly centrifuged. If casein has been added, a gummy mass of undissolved casein is usually obtained. Furthermore, cream containing added casein is in most cases frothy on account of the agitation employed to incorporate the thickening agent.

The ability of casein to absorb a large amount of water finds practical application in its incorporation with sausage meat, particularly meat which has become dry and therefore partially lost its binding power. In Germany the materials used to increase the moisture content of sausage meats have been found to be albuminous in nature, consisting of desiccated egg or serum albumin, casein, or gluten. The objection to this practice is obviously because in effect it substitutes water for meat and greatly lowers the nutritive value of the food per unit weight.

Mansfeld[31] reports the composition of a preparation whose function it is to serve as a binding material in sausage meat. Analysis indicated

that it contained 65.86 per cent casein and 12.24 per cent ash constituents, the latter being largely sodium bicarbonate. Inasmuch as it exhibited a decided tendency to absorb water, it appears that it was well suited for the purposes for which it was intended.

Bakers' Wares

Casein has no general use in the baking industry in the United States except in so far as it is a constituent of the milk used in making breads. To a limited extent it enters into special products intended for dietary purposes. Dry skim-milk has been found to have a beneficial effect upon the volume of the loaf and upon the texture of the bread, when it is incorporated into the dough mix in quantities not exceeding 6 per cent of the weight of the flour used. It is probable that these beneficial effects may be due to some property of the casein.

In the addition of various ingredients it is well to keep in mind the fact that an acid rather than an alkaline reaction is desirable. The former reaction acts as a control upon the fermentation and thereby prevents the development of "rope."

In describing the preparation of an easily digested bread for convalescents, Scherer [32] states that it should possess a light consistency and neutral reaction and be of a high protein content. To that end Hahn and Planitzer [33] prepare a wheat dough in the usual manner and allow it to rise but once. To this dough is then added a soluble form of casein known as "Plasmon" (which contains 75 to 80 per cent of casein, 5 to 7 per cent of sodium carbonate, and 5 to 10 per cent of fat and milk sugar), a rye dough which has not been "set," and a dough made from wheat groats. Bread so made, it is claimed, has all the virtues of pure wheat or rye bread without their disadvantages. Another casein-containing bread is described [34] as being made of a gluten-rich flour, yeast, and water. Following a preliminary fermentation, more of the same flour is added together with salt and casein. The dough is allowed to rise again and then baked.

Schredelseker's [35] baking preparation is made by curdling skim-milk with rennet and incorporating liquid egg yolk with the coagulum.

Egg Substitutes

Mixtures purporting to replace eggs in baking appeared on the market some ten years ago and many exaggerated statements were made regarding the value of these products as economical substitutes for whole eggs. The price of eggs at that time made housewives particularly susceptible to the implied suggestion that a few ounces of this yellow powder represented the nutritive value of two or three dozen eggs. According to

La Wall,[36] no other food substitutes sprang into prominence with such rapidity and with so little merit or legitimate warrant for their manufacture and sale.

Chemical analyses [37] indicated that some form of starch, usually cornstarch and occasionally rice starch, formed the major constituent of nearly every one of these egg substitutes. In addition to the starchy ingredients, one or more of the following were found: casein, gelatin, milk powder, sodium phosphate, sodium chloride, sodium bicarbonate, and in a few cases a little powdered egg albumin and rarely desiccated egg. Baking tests [38] carried out on these so-called egg substitutes showed the superiority of the egg for culinary purposes and the falsity of such positive assertions as "gives better results than eggs," "one scant teaspoon represents one fresh egg," etc.

Legislative action by many states concerning the sale of such preparations proved a deterrent to their acceptance by the housekeeper. Moreover, the federal requirement [39] that the words "egg powder" must not appear upon a product which contains no eggs, and that a product which is labeled to indicate that it will take the place of eggs must possess the properties of eggs, both in respect to food value and baking qualities, eventually made ancient history of a food product, the suppression of whose sale is another milestone in the pure food movement.

A skim-milk product intended for use by bakers as an egg substitute is described by Bernstein.[40] Skim-milk is heated to 95° C., after which flour is added and the casein, containing also albumin, is incorporated with it by precipitation. This procedure seems to have been anticipated by another food product,[41] made from skim-milk casein coagulated in the presence of flour. Upon removal of the whey there is obtained, it is claimed, a light, readily digestible product, which has no great influence on the flavor of breads to which it has been added.

Oleomargarine Manufacture

The problem of so incorporating the ingredients of an oleomargarine that it will respond on heating to the well-known foam test for butter is claimed to have been solved.[42] A nongelatinizing solution of casein, made by heating casein with an alkali bicarbonate and then treating it with an equal amount of sodium chloride, is said to be capable of so emulsifying and binding the fats and water of the oleomargarine that its usual sputtering and grease-like action in a heated pan disappears. It is further stated that the function of the alkali bicarbonate is to cause the foaming and that the appearance of the brown color is due to sugar added as such or through the medium of the casein used. Although these claims can probably be accepted with some reservations, the use of casein

in this direction apparently has its American counterpart in the practice of adding milk to oleomargarine not only for whatever of nutritive value may be gained thereby, but also because of the emulsifying properties of casein and the other constituents of milk.

Monhaupt's [43] milk-like solution, prepared by mixing finely comminuted casein with magnesium oxide and water, is said to be suitable for use in foods and confections. A strong solution so prepared may be used, it is claimed, as a substitute for white of egg or, with the addition of fat, as a "cream" and in that form it is further said to be suitable as the major ingredient in the manufacture of an artificial milk and an oleomargarine.

Ice Cream Filler

Skim-milk powder emulsified with butter fat is widely used in ice cream. Letters patent have been granted Turney [44] on the use of casein as an ice cream filler. To that end the swelling of the casein in the milk used is induced by pepsin, rennet, or acid. The swollen particles of casein are then "semidissolved," it is stated, by the addition of lime and sodium bicarbonate. Sugar is also added.

Soup Tablets

Soup tablets (Suppenwürzen), bouillon cubes, and similar forms of concentrated foods for preparing soups with a minimum of effort consist only in part of meat extractives. The ingredients, of course, are varied to suit the particular formula of the manufacturer. In Germany it is the practice to use casein as the major constituent of the fluid and semisolid products of this type.

Miscellaneous Uses

A casein phosphate preparation made from precipitated casein and phosphoric acid or monocalcium phosphate finds use, on desiccation, as a constituent of baking powder.[45] Soluble "casein sodium" is sometimes added to cocoa, and a readily soluble and assimilable product is made from casein by the admixture of common salt, the mixture being heated to about 110° C. in order to induce, it is claimed, hydrolysis of the former.[46]

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Chapter 10

Miscellaneous Uses of Casein, Statistics; and Bibliography

Besides the subjects which have been discussed at length in the foregoing chapters there are listed in the trade and chemical journals many other uses of casein, among which may be mentioned the following:

Preparation of pastes.
Leather dressing and finishing.
Soap manufacture.
Cask glazing.
Making picture moldings.
Shoe polishes.
Photographic plates, films, and printing papers.
Thickeners in textile printing.
Oil cloth and linoleum.
Composition cork.
Cementing liners in bottle caps.
Paint removers.
Reducing flammability of celluloid.
Spreaders for insecticide sprays.
Imitation leather.
Clarifying wines.
Solidifying mineral oils.
Boiled oil substitute.
Stenciling paint.
Preparation of colored micas.

In going over the literature on casein it has been found that many of these supposed uses consist only of the descriptions given in patent specifications, and in most cases it has been impossible to find out whether they have ever been applied commercially. While it is possible that some of them may have been of local or temporary importance, it is very evident that they have not been developed to any marked extent and that they consume only a very small part of the total casein made. While investi-

gation of a number of the uses listed has been entirely without results, it has proved possible to obtain a little information along a few lines.

Photography *

Casein has been proposed for use in sensitizing solutions, for making casein-pigment prints, in printing out papers, and for films. Its use has apparently never gone much beyond the experimental stage and about the only information available consists of the following patent specifications:

British patent 19,297 (Sept. 14, 1908).
British patent 12,887 (1905).
German patent 202,108 (May 15, 1907).
British patent 20,740 (1907).
French patent 151,014.

Soaps

It is claimed in *Parfumerie Moderne* for September, 1918, that while casein in soap does not add appreciably to its detergent power, it does have a favorable action on the skin. It is said to give an agreeable texture to the soap and frequently to improve the bad odor of the fat. In neutral or slightly acid soaps, however, it tends to provoke rancidity. In preparing the soap the casein is dissolved by means of borax and soda and added to the hot soap before it goes to the frames. It appears that it is sometimes used in so-called "milk soap" or "buttermilk soap" but it probably adds nothing to the value of the soap, though it may maintain a semblance of honesty in the product.

The small part played by casein in the soap industry is evidenced by the replies of three important manufacturers who were asked for information: of the three, two did not use it at all, while the third used it only occasionally.

Casein in the Leather Industry †

The use of casein in the leather industry dates back for thousands of years to the prehistoric days of tanning, only in early times it was used in the form of milk while now it is employed as a solution containing just enough alkali to dissolve the casein completely. The alkalies used are generally borax and ammonia, though caustic soda, sodium carbonate, or trisodium phosphate will serve equally well if they are not used in excess.

The function of casein in leather manufacture is that of a finishing

* Courtesy of Dr. S. E. Sheppard, Eastman Kodak Co., Rochester, N. Y.
† By F. L. Seymour-Jones.

or seasoning material. Seasoning is about the last operation performed on leather before it leaves the factory to be converted into leather goods. It consists in the application of a solution to the grain surface (the exterior of the skin as it comes off the animal) which serves to give the surface a certain gloss. This is generally enhanced by the use of a glazing machine which consists of a cylinder of glass, mounted on a mechanically driven arm, which passes with adjustable pressure over the skin held on a leather strap which lies on a wooden bed. The friction of the glass on the thin film of casein, or other seasoning material, develops the gloss. Although the finish is applied after the dyeing of the leather, dyes are often mixed with the seasoning to intensify or modify the shade, a process which is known as "flaming."

Besides casein, seasoning materials include such diverse substances as blood and egg albumens, Irish moss, tragacanth and other gums, glue, gelatin, shellac, etc. The function of the proteins differs somewhat from that of the mucilages and gums. The latter serve more to fill the leather and cover up defects, while the proteins act as a glaze on the surface. Of the proteins casein does not give such a high lustre as blood or egg albumen, but it helps to hold the gloss because of its waterproof qualities.

Casein finishes are applied to light leathers, such as sheep skins, to heavy grades of stock, and to heavy splits of cow hide. Sheep skin for the sweat bands of hats has been successfully finished with finishes made in part from casein, although the stretch of this class of skin does not make it so satisfactory for the rather hard finish produced in this way. In order to overcome the hardness and brittleness of casein finishes it has been found desirable to use some material such as glycerol or Turkey red oil to improve their elastic qualities. On heavy stock, such as case leathers and bag and strap stock, casein finishes are especially satisfactory and yield remarkable waterproof results which become more pronounced as the leather ages.

One of the most extensive uses for casein in the finishing of leather has developed during the past decade. In this period wide use has been made of what are known as "pigment finishes," in which pigments of suitable colors are mixed with the other ingredients of the finish before it is applied to the leather. The earlier types of this finish depended on glue for a binder, but these were not satisfactory because of their inability to withstand water or excessive moisture. Casein, because of its more waterproof quality, has very largely supplanted glue in the manufacture of these finishes and has proved very satisfactory, especially when used with such materials as carnauba wax, beeswax, stearic acid or blood albumen to aid in obtaining a high gloss.

For judging the value of casein as a seasoning Harvey [1] recommends the determination of moisture, ash, fatty matter and proteins. Excessive

fat, poorly emulsified, is liable to cause an uneven glaze. The casein should also be completely and readily soluble when the correct amount of alkali is used. There are on the market soluble caseins, generally consisting of casein mixed with borax or borax and sodium bicarbonate. Usually it is preferable to purchase casein and solvent separately and combine them in the desired proportions, as in this way the consistency of the finish may be better controlled.

The use of casein for decolorizing tanning extracts has been suggested, but is not yet perfected. Albumen solutions are frequently used at present, being added to the tanning extract, which is then heated. The coagulated albumen settles out, carrying with it the undesirable coloring matters. Since casein is coagulated by acid, the tanning extract would cause its precipitation, and it would then exert a decolorizing action in the cold. Casein dissolved in alkali would, however, probably neutralize the natural tannin acidity too far, although it could be used in the form of skim milk without injurious effects.

Textiles *

Casein is not extensively used in the textile industry, although certain of its properties render it adaptable for various processes of textile printing and finishing. The value of its application lies in the fact that casein may be precipitated from solutions of certain caseinates, particularly those of sodium and ammonium, and rendered still further insoluble by the action of formaldehyde.

It is in the pigment style of calico printing, in the preparation of double cloths as an adhesive, and in the finishing and waterproofing of cotton material that casein has found its most extensive uses. It has also been suggested at different times that cotton material treated with casein might become sufficiently "animalized" to develop an affinity for acid dyes, similar to that of the animal fibers. Such a process does not appear to have been worked out at any time with sufficient success to have warranted its adoption by the trade.

An alkaline solution of casein has also been suggested as an aid to detergent action, but for this purpose it apparently has no advantage over the ordinary soaps, either as to cost or effectiveness.

Ever since the earliest days of calico printing, the so-called pigment style has been used. Through the adoption of this style, it is possible to print upon textile material various pigments and color lakes in conjunction with some adhesive substance, which is of a more or less soluble nature while in the printing paste, and yet capable of being converted into a decidedly insoluble substance later. Albumin has always furnished

* By Prof. L. A. Olney.

a satisfactory substance for this purpose and being soluble in cold water it could be readily incorporated in sufficient quantity into the printing paste, together with a colored mineral pigment. After printing this color paste upon the cloth, a passage through a steam ager coagulates the albumin and a colored print results which possesses fastness to washing and light which are satisfactory for all ordinary requirements.

It is as a substitute for albumin that casein has been chiefly used in textile printing. The pigment to be used is incorporated with the casein which has been dissolved in a warm, slightly alkaline solution, and with the addition of proper thickeners and gums this may be made into a paste suitable for textile printing. The printed material is then dried and passed through a solution of formaldehyde or through a steam ager containing steam and formaldehyde vapors. In this way the casein is rendered sufficiently insoluble to hold the pigment upon the fiber. When a fabric printed with a pigment and casein paste is submitted to formaldehyde vapor alone there is danger that only a surface combination will be made, whereas if the formaldehyde is introduced directly into the paste a more permanent fixation will result. It requires considerable experimentation and skill, however, to do this in such a way that the coagulating effect will not take place until the goods are steamed or exposed to the air for some time. The addition of hexamethylenetetramine to the casein printing paste is said to be beneficial. Prints produced in this way are not as fast to an alkaline soap washing and not as resistant to various other reagents as albumin prints. This, together with the fact that the use of casein involves more work and complications, has prevented it from making any great headway in replacing the albumin method.

In the allied industry of wall paper printing, which is quite closely related in certain respects to textile printing, mineral pigments and color lakes have been successfully printed using casein with the addition of a small amount of formaldehyde to the color paste just before printing.

As a finishing material casein has a tendency to make the goods rather too stiff, but for the finishing of light weight materials, such as organdies, it has been used with some success.

A somewhat different use of casein in the textile printing industry has been in connection with color furnisher brushes. The brushes are immersed in a solution of casein and formaldehyde and the former becomes fixed upon the fibers, either through an air drying or steaming. The surface of the fibers of the brushes is thus protected, and becomes more durable.

In the manufacture of double cloths where a piece of rather firm material like duck or heavy twill has to be backed by a softer fabric, as, for instance, cotton flannel, casein has been used to some advantage as an adhesive in holding the two fabrics together. For this purpose casein

is commonly dissolved with a mild alkali, as, for instance, borax or ammonia. The latter has the advantage of evaporating during the process of drying and thus aids somewhat in developing the insolubility and adhesive character of the casein. A small amount of castor oil, or some other oil softener, added to the casein solution improves the pliability and softness of the finished fabric.

In the preparation of material waterproofed with casein the cloth is first prepared with a solution of some aluminium salt, preferably the acetate, and thoroughly dried. The cloth thus prepared is then passed through a solution of casein in ammonia and a little soap, and then again dried. A final passage through another solution of an aluminium salt, as, for instance, alum, is also an advantage. In this way a combination of aluminium soap and aluminium caseinate is formed which produces a shower proof effect upon the material.

In the manufacture of emery cloth casein has been found to be satisfactory for backing the cloth before applying the glue.

Casein in Insecticide Sprays *

Within a comparatively few years casein has found quite a wide application as a spreader for spray materials as applied to fruit trees, vegetables, etc. It is a common observation that poisons applied as a spray without a spreader tend to roll off the surface of many kinds of foliage and fruit, and that the spray runs off the upper surfaces of twigs and branches, leaving them insufficiently covered. The thoroughness of application of such a spray is judged largely by the number and proximity of the spots of the dried spray material, and it is perfectly obvious that there are many interspaces which are apparently free from poison. This condition tends to limit the effectiveness of sprays and much study has been given to improving their physical properties so that better results could be obtained.

Robinson [2] states that an ideal spreader should cause a continuous film of poison to be deposited on the surface; it should increase adherence; it should not react chemically with the poison to form by-products which would cause foliage injury; it should not diminish the toxic properties of the poison; and it should not cost too much. While it may be impossible to find a spreader which will fulfill all of these requirements it has been found that many substances effect great improvements over the old types of spray. Such substances may be divided into two classes; namely, the soaps, and the materials containing water soluble or colloidal solutions of proteins. In general the soluble proteins are effective at much lower concentrations than the soaps.

* By E. Sutermeister.

Of the proteins casein has been much the most generally used. The first work of any importance with this material was carried out by the late Professor A. L. Lovett [3] of the Oregon Agricultural College, who made orchard observations of the spreading qualities of various materials in 1919. Since that time its use has increased greatly until its estimated consumption in 1925 was 300,000 pounds, with prospects of a considerably larger use in the immediate future.

There are several commercial brands of casein spreaders now on the market, most of which consist of finely ground casein mixed with about three times its weight of dry hydrated lime. This is convenient to use but if desired an equally satisfactory mixture may be made by the consumer, the only requirements being that the hydrated lime shall be dry and finely divided, and that the casein shall be of good quality and shall be ground at least fine enough to pass a 100-mesh screen. Such mixtures of the dry materials may be dissolved in a number of ways, all of which are designed to avoid the formation of lumps. It may be washed through a strainer into the sprayer tank while filling the latter with water; or it may be beaten into a small amount of water, much as flour is mixed, and subsequently diluted; or it may be shaken in a closed receptacle in the proportion of one pound of spreader to a gallon of water. Equally satisfactory results may be obtained by mixing 4 ounces of hydrated lime to a smooth paste with about an equal amount of water, diluting to one pint and stirring in 4 ounces of casein. Stirring must be vigorous and continued for at least five minutes, or until a thick, fluffy paste has been obtained; when diluted with an equal volume of water this is ready to add to the other spray materials.

Spreaders prepared with lime possess the desirable property of drying to a practically insoluble film, which means that the poison is not easily washed off the fruit or foliage by rains. Where this property is not of such great importance a spreader may be made by dissolving the casein by means of sodium hydroxide, sodium carbonate, or sodium bicarbonate. Dissolve the sodium hydroxide, or other alkali, in about a quart of water and sift a pound of the casein slowly into the solution, stirring meanwhile. Continue stirring until the casein has entirely dissolved, and add water to any desired consistency. This solution may be used as a spreader in the same concentration as the lime-casein; namely, about three-quarters of a pound to 100 gallons of spray. For this spreader a less soluble and coarser casein may be used, since the solvent power of the various compounds of soda is greater than that of hydrated lime.

The amount of lime-casein spreader to use is variously stated as three-quarters of a pound to two pounds of the dry mixture for 100 gallons of spray. The smaller quantities are apparently used with lead or calcium arsenate while the larger amounts are recommended for oil

sprays. It is also used with Bordeaux mixture, nicotine sulfate, lime-sulfur and other materials. To secure the best results many factors, such as the type of gun or nozzle, the kind of surface to be covered, the atmospheric or climatic conditions, etc., must be taken into account and the amount of spreader governed accordingly. Most of these points need to be settled by careful field observations before the general principles can be established with certainty.

A satisfactory oil spray may be prepared by dissolving 1 pound of spreader in water, making up to 4 gallons in the sprayer tank and adding 8 gallons of oil. The pump is then started and the mixture sprayed back into the tank at a pressure between 200 and 300 pounds until a good emulsion is obtained. A stock oil emulsion may be prepared from 50 gallons of oil, 25 gallons of water, 4 pounds of spreader and half a pound of copper sulfate. Dissolve the spreader in a little water and add the rest of the water, add the copper sulfate dissolved in a little water, and finally add the oil. Pump back into itself at high pressure until a good emulsion is obtained. In all such mixtures the spreader should never be added to the oil, but should be dissolved in water first.

The advantages claimed for lime-casein spreader are very numerous, among them being that it coats all parts of the twigs and foliage thoroughly; dries quickly; will not wash off with rain; reduces sunburn; avoids spotted fruit; permits fruit to color evenly; overcomes spray burning; keeps spray nozzles from clogging; keeps materials from settling in the spray tank and makes the spray more conspicuous so that covering can be observed better. Making due allowance for the enthusiasm of advertisements, most of these claims seem reasonable and many are vouched for by the tests at various Experiment Stations.

After it was observed that solutions of proteins spread well on most surfaces the study of this class of substances was extended to include albumin, pepsin, hot water extracts of dried blood, sage, clover, vetch, alfalfa, etc. These were found to spread well on all surfaces tested, thus adding to the evidence that soluble protein is the active agent. Another possible substitute for lime-casein spreader is skim-milk or some of its products, as dried or condensed skim-milk. Tests in the laboratory and a few field experiments indicate that 2 or 3 quarts of skim-milk in 100 gallons of spray will give good results on many surfaces including the foliage of apple, pear, prune, peach, cherry, and cabbage, and apple and pear fruits. Equally good results were obtained with the dried product in equivalent amounts. With all skim-milk products it is necessary to add a few ounces of hydrated lime to each quart of milk, or its equivalent; if this is not done spreading will not result. These milk products are easily brought into solution and in this respect are preferable to commer-

cial lime-casein, which requires much more careful handling in order to give satisfactory results in the sprayer.

Probably the most important point to consider in regard to spreaders is the cost. If this is low spreaders can be used to good advantage in most sprays, but if it is high they should be used only in those cases where the benefit derived is sufficiently great to justify the expense.

Statistics.

It has proved quite impossible to obtain any subdivided statistics regarding the uses of casein, so any statements regarding the quantities employed in the different industries should be accepted as merely estimates.

According to the U. S. Department of Commerce the production and imports of casein, and the imports of casein products, particularly Galalith, are as follows:

Produced in the United States.

	1914	1922	1923
Dried casein, Skim-milk	18,570,220	6,907,000	14,500,000
" " Buttermilk	.....	20,000	48,000

Imports.

	1913	1922	1923	1924
Pounds	8,805,891	14,342,498	26,489,992	17,749,985
Dollars	649,371	1,367,828	4,409,744	1,384,661

Imports of casein products, particularly Galalith.

		1913	1922	1923
In blocks, sheets, rods, tubes or other forms.	Lbs.		31,954	188,553
	$		14,251	81,872
In finished or partly finished articles	Lbs.	12,764	9,443	90,191
	$		19,948	214,489

For the year 1923 the importing countries, with the amounts of casein which they supplied, were as follows:

Country	Pounds	Country	Pounds
Denmark	25	B. C. and Yukon	1798
Finland	187,878	Bermuda	56,000
France	1,835,139	Argentine	19,198,023
Germany	360,731	Brazil	14,220
Italy	20	British India	425,451
Netherlands	44,092	Hong Kong	80
Norway	49,890	Switzerland	121
England	821,608	Australia	1,084,418
Quebec and Ontario	11,501	New Zealand	2,407,942
Canada, Prairie Prov.	55		
		Total	26,489,992

It is estimated by the Department of the Interior, Ottawa, that the plywood industry in the United States used about 7,000,000 pounds of

casein in 1922. It has also been estimated that about 75 per cent of the domestic casein is used in the coating of paper.

In 1922 there were said to be 74 producers of casein in the United States, while in 1923 this had risen to 92.

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* Contains information on casein glues.

Patent Literature on Casein Glues

U. S. Patent	838,785	Isaacs, 1906
	845,790	
	848,746	
	183,024	Ross
	21,744	Gardner, 1896
	609,200	Hall, 1896 (Reissue 11, 811, 1900)

A large number of articles have been published on the chemical properties of pure casein. They can be located through "Chemical Abstracts." Most of the articles published in English have appeared in the following journals:

Journal of Biological Chemistry
Journal of Physical Chemistry
Journal of the American Chemical Society
Technical Bulletins of the New York Agricultural Experiment Station.

Chapter 11

Storage of Casein

By A. H. Warth

Commercial casein is a commodity which requires much of the attention given to a food product in the way it is handled in packing and in storage. It is not more perishable than a dry food product, but requires far more attention to storage conditions than does gelatin glue if it is to be kept free from infestation by mites, vermin and rodents. Therefore, prime consideration must be given to the place and conditions under which it is stored, especially if it is to be kept for an extended period of time, such as six months or more.

Life of Product.

Casein which has been properly prepared with regard to cleanliness may be kept for two and a half years, or over two successive summer seasons, provided it has been packed in clean, closely woven, burlap bags, and properly stored.

Warehouse Conditions.

The warehouse in which casein is stored should be clean and dry, and maintained at a temperature below 80° F. The floor should be one which is sufficiently elevated to prevent danger of ground moisture and it should be swept frequently and kept in clean condition. If packaged casein is piled on skids, the latter should be whitewashed, or painted with cold water paint, and the walls should also be kept painted. It is essential that the warehouse room receive ventilation, but such ventilation, if from windows, should be through screens, and the entrance door should also be screened.

Where large quantities of casein are stored in the original bag or sack containers, it is important to separate shipments into different lots and pile the lots apart from each other. If possible these piles should be so arranged as to insure the best ventilation from the source of fresh air supply. When there is little or no circulation of dry air in a warehouse, foul air pockets will build up in the pile or in the proximity of certain of the piles. Measurements of temperature and relative humidity

of the piles show discrepancies which can be accounted for only by lack of circulation. The following table illustrates the wide variation possible.

	Pile 1	Pile 2	Pile 3	Pile 4
March 21, 1921....	Temp. 64° F. R.H. 66%	Temp. 63° F. R.H. 65%	Temp. 60° F. R.H. 63%	Temp. 63° F. R.H. 74%
March 23, 1921....	Temp. 54° F. R.H. 48%	Temp. 54° F. R.H. 48%	Temp. 53.5° F. R.H. 53%	Temp. 54° F. R.H. 48%
March 24, 1921....	Temp. 53.5° F. R.H. 76%	Temp. 53° F. R.H. 75%	Temp. 54.5° F. R.H. 65%	Temp. 53° F. R.H. 75%

Of these piles, numbers 1 and 2 received a little ventilation from one door and this air was not dry. The other piles received less ventilation. A relative humidity of 65 per cent or below is best for the storage of casein and the use of air which has been dehumidified by a regular dehumidifying equipment is ideal, especially if large tonnages are to be stored. Where the loading or removal of bags is conducted from the front end of a warehouse the storage is best located in the rear. At this end an exhaust fan blower may be placed and kept operating except when outside atmospheric conditions are unfavorable.

Even under ideal warehouse conditions, it is unsafe to assume that any quality of casein can be favorably stored. One questionable lot of casein placed in the proximity of a lot of exceptional purity may cause the ruination of the good lot. The purity, or cleanliness of the casein has a definite relation to the life of this commodity, as will be referred to later; therefore it is evident that, aside from examining a casein for its solubility and glue strength, a knowledge of the purity of the casein is also essential. Casein prepared from whole milk instead of skim-milk, from dried curd which has become mildewed, or from improperly washed or dried curd may have very poor keeping qualities.

The importance of observing minor details concerning storage should not be overlooked, as when casein becomes infested, the infestation spreads with such rapidity that losses of serious proportions are incurred, especially where casein is stored in carload lots. Large users, as well as suppliers, have at times experienced such losses running into many thousands of dollars.

Packing Methods.

Until recently American manufacturers have not learned the significance of details to be given to the packing of casein, although for many years Argentine casein has been imported in packages that are close to the ideal. Argentine casein is packed in closely woven burlap bags holding 60 kilos, and tightly stitched so as to avoid folds and crevices. Domestic caseins are now being packed in closely woven sacks averaging 125 pounds

gross, and measuring 3.7 cubic feet in contents. Under no circumstances should casein be packed in second-hand bags, even though they are washed and renovated, nor should casein be packed in coarsely woven new bags of any kind. This applies particularly to the ground product. It is always preferable to double stitch the bags with stout twine.

Bin Storage.

Large users of casein occasionally find it necessary to empty casein upon receipt into a bin, from which it is withdrawn as required. There are commercial advantages in handling casein in this way, and often bin storage is used for the purpose of blending two or more varieties. While this method of handling has advantages it cannot be wholly recommended, as the product may become infested with insects without sufficient warning to permit of checking the infestation except by drastic means.

If it is necessary to employ bin storage, the bins should be constructed with sloping side walls so that the casein will not pocket and will be kept freely moving. Bins are best made from tongue and groove maple, where wood is used, or they may be sheeted with metal, such as charcoal tinplate, when built of ordinary wood. They may be filled from openings in the floor above, and the casein removed by means of a screw conveyor located at the foot of the sloping bottom of the bin.

Infestation.

Casein is subject to infestation by molds, bacteria, insects, and rodents. The latter are often a serious source of annoyance as they gnaw the bags, spilling the contents and subjecting it to the danger of insect infestation. They also occasion losses by what they actually consume and by making the spilled casein so dirty that it is not fit for use.

Molds.

Casein is most often contaminated by mold spores while in the state of moist curd during manufacturing, but any casein which has become slightly moistened or wet furnishes a good food supply for all types of mold. Since the air is filled with spores of various kinds it is easy for them to gain access to stored casein and if sufficient moisture is present they will immediately germinate and multiply. If the casein is kept dry the spores will not develop and if a casein is mold-free when packed it will not become moldy in storage, unless it becomes moist and is infected by spores from without. A wet bag of casein, or one which shows signs of having been wet in transit, should never be stored in the same warehouse room or bin with other lots of casein.

The number of molds per gram of casein varies greatly according to the conditions of manufacture; a California casein possessing a strong sour odor contained 500, while a natural soured casein contained 1000

molds per gram. The molds were mostly of common types as mucor, aspergillus, and penicillium, all of which cause decomposition of casein.

Moths.

By far the most common and dangerous infestation of casein is by a small moth. At first little was known concerning the nature of this moth and it was a constant source of annoyance to both casein producers and consumers. Its ravages have at times been the cause of the loss of an immense tonnage of casein in the United States.

E. A. Back, Entomologist in Charge of Stored Product Insect Investigations, U. S. Bureau of Entomology, Washington, D. C., has identified the moth commonly infesting casein as the webbing clothes moth, Tineola biselliella. He states that it is peculiar that this pest, which normally feeds upon woolens and furs, should also infest casein. Mr. Back further states that a thorough fumigation with hydrocyanic gas will prove very satisfactory as a control measure.

Frank E. Lutz [1] describes the moth as follows: Tineola biselliella has a webbing larva; it makes no case but feeds naked, usually in a fold or crevice of the material it is eating, and often under the web of silk which it spins wherever it goes. The cocoon is an irregular affair of silk and food material, somewhat resembling the case of pellionella, another species of clothes-moth. The adult is about the size of pellionella. The front wings are yellow and without spots; the hind wings are pale.

The other species of moth known to infest casein is Tinea pellionella. Tineola biselliella is the commonest of the clothes-moths from Pennsylvania southward, while in the northern portion of the country Tinea pellionella is much more common. This latter moth is darker colored than the former and the larvæ spin movable cases in which they travel about. In the warmer parts of the country it is many brooded and the processes of generation go on continuously, while in the north cold weather arrests them.

When these insects manifest themselves in commercial casein they are often found in quantities, clinging to the inner side of the burlap bags, and also at the top and bottom of the product. If one bag in a warehouse becomes infested all bags in the proximity are also likely to become infested, as the full grown moth, freeing itself from the mesh of the bag, will fly to another bag, deposit its eggs, and the larvæ resulting will go through the cycle to the adult moth.

Beetles.

Several kinds of beetles and their larvæ are known to infest casein. They are numbered among the "stored grain" and "leather" pests. The

more common of the "stored grain" pests to infest casein are the cadelle (Tenebrioides mauritanicus), the foreign grain beetle (Cathartus advena) and the confused flour beetle (Tribolium confusum). Of the "skin-devouring" species which infest casein the larder beetle (Dermestis lardarius) and the leather beetle (Dermestis vulpinus) are undoubtedly the most common.

The cadelle is a slender, flattened, black beetle, about one-third of an inch long. It resembles the meal worms in appearance but is smaller, and the larvæ are dirty or chalky white in color and relatively longer than those of similar beetles. The foreign grain beetle is a small, reddish brown beetle, and because of its relatively small size it often remains undiscovered in the warehouse. It feeds mostly on mold, to which it is readily attracted. It does not infest casein unless the latter has become moldy, and it will not inhabit a place which has been well whitewashed. The confused flour beetle is often a serious pest. It is a slender, dark brown beetle, scarcely one-sixth of an inch long. Instances are known where these beetles have infested casein to such an extent that they have literally covered the bags on the outside, and the burlap resembled the top of a pepper shaker because of the holes where they emerged.

Dermestidæ are usually oval, plump, dark beetles, less than three-eighths of an inch long. In Dermestis vulpinus there are two characteristic white spots below the last abdominal segment. Dermestis vulpinus is more apt to be encountered in the south than Dermestis lardarius. None of the beetles do nearly so much damage as the larvæ of moths.

Biological Tests and Standards.

Biological tests have been neglected to a sad degree in determining the cleanliness in the preparation of so important a commodity as casein, and much more attention should be paid to them. This field has been less neglected by the gelatin glue makers, and when followed is capable of giving the greatest economic results. Biological tests on casein are of importance to the producer, vendor and purchaser, as a means of establishing quality grades, as well as preventing subsequent deterioration of the quality of the casein stored. Caseins may be judged biologically by both the micro-organism count and by the incubation test, and standards established which will insure good commercial quality and satisfactory operating results in all of the various lines of industry which employ casein.

In formulating biological standards for casein, consideration may be given to the specific use to which the casein is put. In the manufacture of pastes, food products, composition cork, cold water paints, casein latex compositions, etc., it is quite essential to hold to a high standard for the purpose of maintaining uniform quality of production. In the

manufacture of Karolith, or any of the hard plastics, it is not so essential; and in mills where the casein is washed, as in certain lines of coated paper work, the impurities are more or less removed and the washed product may conform to its required standard.

The following standards are drawn for such commercial caseins as are used in pastes, adhesives, and coatings of the higher grade. They are also applicable to food caseins and to grades of caseins that are to be kept for an extended length of time.

Caseins containing 100 or less molds per gram, but decomposing in 24 hours under incubation are rejectable.

Caseins containing more than 100 molds per gram, even though decomposition in incubation does not set in within 72 hours, are rejectable.

Caseins containing larvæ, webs, or moths of any species are rejectable.

Caseins acceptable for storage are those that contain 100 or less molds per gram and which do not decompose within 72 hours.

Micro-organism Count.

A quantitative estimation of the number of micro-organisms in casein may be made by colony enumeration in plates of agar. In conducting this test it is absolutely necessary that all glassware used shall be sterile, which may be assured by keeping it at a temperature of 150° for thirty minutes in an oven. After all glassware has been sterilized, 10 grams of casein are weighed out and enough boiled, cooled, distilled water added to make up to a volume of 100 cc. in a sterile bottle. After thoroughly mixing, shake the bottle at least twenty-five times, then withdraw 1 cc. of the supernatant liquid with a sterilized pipette and deliver it into a sterilized Petri dish, 10 cm. in diameter. To the liquid in the Petri dish add 10 cc. of standard nutrient agar solution at a temperature of 40° C. After tipping the dish back and forth a few times to mix the contents allow the agar to solidify in a horizontal position, transfer the Petri dish to an incubator as soon as the agar is hard, and incubate the culture in a dark, well ventilated incubator where the atmosphere is saturated with moisture. At the end of twenty-four hours count the colonies with a lens which magnifies five diameters. The number of colonies counted, multiplied by ten, gives the number of micro-organisms (bacteria or yeasts) contained in 1 gram of the sample of casein. If the micro-organisms are molds they will require several days for development.

Warth Incubation Test.

A 200-cc. clean Florence flask, and a 4-gram plug of cotton on a watch glass, are placed in a drying oven for 2½-3 hours at a temperature of 120° C. in order to sterilize both flask and cotton. The plug must be

of sufficient size to supply enough friction when placed in the mouth of the flask to permit the latter to be lifted by the plug.

Fifteen grams of the sample of casein are placed in the flask, and 90 cc. of recently boiled and cooled distilled water are added. The flask is stoppered with the cotton plug, and, without shaking the contents, is placed in an oven maintained at a constant temperature of 38° C.

It is left in the oven until putrefaction sets in, as is evidenced by a distinctly disagreeable odor often accompanied by fungus growth. The plug of cotton may be taken out at intervals in order to note the progress of putrefaction. A good product will stand up well for at least 48 hours and will show no signs of marked decomposition under 72 hours.

A casein which will hold up for 72 hours is one which may be stored in a dried state for more than a year, or even two years, without deterioration of its chemical or physical properties. Caseins which "go bad" under the test are generally those which have been insufficiently washed, insufficiently dried, or otherwise improperly prepared. Such caseins can only be stored with considerable risk of deterioration and insect infestation, which may occur within the course of a very short time.

Results of Examination of Commercial Caseins under Warth Incubation Test:

Sample No.	Kind of Casein	Time Required to Putrefy	Remarks
2283	British Indian	72 hours	Mold Fungus
2300	U. S. Hydrochloric	36 hours	Putrefaction
3023	Ditto	16 hours	Ditto
10628	Argentine Hydrochloric	24 hours	Moisture 9.3 per cent
2284	U. S. Sulfuric	Over limit	No change in 124 hours
2285	Ditto	Ditto	V. Sl. change in 124 hours
2301	Ditto	Ditto	Held 110 hours
2312	Ditto	36 hours	Putrefaction
3843-9	U. S. (5 samples)	60 hours	Ditto
9751	U. S. Lactic	72 hours	Decomposed in 96 hours
3556	Argentine Lactic	Over limit	Held over 96 hours. Bacterial count less than 100 per gram
10641	Argentine Lactic	24 hours	Moisture 10.2 per cent Mold Spores—9000 per gram
14973	Ditto	48 hours	Original sample had musty odor
15063	Ditto	60 hours	Badly decomposed
15164	Ditto	Over limit	Original sample very clean
2312	French Lactic	Ditto	No change in 110 hours
15101	New Zealand Lactic	40 hours	Unclean bags developed moths
15267	Ditto	48 hours	Developed moths in 6 months
15274	Ditto	Over limit	Slight change in 72 hours

In addition to the above, results of incubation tests on numerous samples of lactic caseins (mostly Argentine) showed no signs of putrefaction within the prescribed time limits. It was also found that these caseins did not deteriorate appreciably in two years' storage.

Bacterial Count *vs.* Incubation Test.

It has been found that the time required for a casein to fail on incubation test is not necessarily a function of the number of bacteria in the sample, even though but one general type of casein, such as lactic acid precipitated casein, be considered. The following table by C. L. Ewing gives the bacterial count per gram and the incubation tests on four samples of casein.

	Bacteria per gram (Approximate)	Incubation Test
Sample 1.....	4,000	Decomposed in 10 days.
Sample 2.....	4,000	" " 6 "
Sample 3.....	2,000	" " 7 "
Sample 4.....	10,000	" " 10 "

The type of bacterium plays a more important rôle in the decomposition of casein than does the actual number. A casein which will stand up only from twenty-four to forty-eight hours on incubation is generally one which contains a large number of a type of liquefying or decomposing organisms. The presence of ten thousand bacteria in a gram of casein does not indicate any serious contamination, as milk normally contains that many or more.

Changes in Casein During Storage.

An Argentine lactic casein stored in a closed 60-kilo sack in a dry warehouse at a temperature ranging between 15° and 30° C. showed a loss in viscosity from 55 seconds to 50 seconds in the course of 18 months, and a corresponding loss in glue strength. A New Zealand casein, which in 6 months' storage became infested with moths, showed a decrease in viscosity from 58.5 to 41 seconds. The viscosity of both of these caseins could be increased by sifting out the fine casein dust resulting from the deterioration of the product. The viscosities here referred to were determined by means of an Engler viscometer, on a 10 per cent solution of a sample dissolved in water with 15 per cent of its weight of borax. The times of flow are for the discharge of 100 cc. of the solution at 70° F.

The following viscosities and glue strengths were obtained on Argentine lactic caseins held in storage for a year and a half, the stock being fumigated by sulfur dioxide after deterioration had set in:

Sample No.	Viscosity	Glue Strength
484	37.8 sec.	795 lbs. per sq. in.
495	42.0 "	975 lbs. per sq. in.
413	45.5 "	932 lbs. per sq. in.

The original standard for the three lots was 45 sec. viscosity and 1000 lbs. per sq. in. glue strength. Sample 484 was found to have become both rancid and sour, while 495 and 413 were perfectly normal in chemical composition.

The amount of borax required to neutralize and dissolve a casein bears a definite relation to the age and condition of the casein. This, and also the loss of strength which casein undergoes when subjected to unfavorable storage conditions, is shown by the following storage records for four samples.

In October, 1911, four samples were stored in a room which was warm in winter and hot in summer. Samples A and B were full bags but of unknown kinds; samples C and D were small lots in cloth bags, C being a domestic sulfuric and D a domestic muriatic casein. These were tested for the amount of borax required to give a neutral solution, and for the amount of casein required to give a full strength coating with 100 parts of clay when applied to paper. When the tests were repeated at intervals the following results were obtained:

Per Cent Borax to Give Neutral Solution

Casein	A	B	C	D
Oct. 1911	10	17	7.5	6.5
Apr. 1912	9.5	16	6	5.5
Oct. 1912	7.5	14	6	4

Parts Casein per 100 Parts Clay in Coating

Oct. 1911	7	8-9	8	7
Apr. 1912	8	8-9	8	7
Oct. 1912	10	12	8-9	8-9
Apr. 1913	8-9	12	9	8-9

In taking the samples for tests from A and B the outer portion was avoided where it had become wormy, but because of the small size of lots C and D this could not be done and they were thoroughly mixed before sampling. All lots appeared in good condition in October, 1911, and in April, 1912, but worms had made their appearance in October, 1912.

A Scandinavian casein stored in June, 1911, and examined in the winter of 1912-13 lost 3.5 per cent of its weight in the form of webs and adhering casein when it was screened on a 15-mesh wire. The strength test mentioned above showed that 10 parts of the sifted casein were equal to 11-12 parts of the unsifted.

An unground domestic lot was screened and 7 parts of that retained on the 15-mesh screen found to have as much strength as 12 parts of the portion passing through the wire.

Another domestic casein gave strength tests of 10 parts of the retained to over 22 parts of the sample passed by the screen. In the latter case

the solution was almost water thin and contained considerable insoluble matter.

In a single lot of casein which tested 9 parts per 100 of clay in October, 1910, the part retained by the screen in the winter of 1912-13 tested 10 parts per 100 clay while the portion passed by the screen required 24 parts. This was for the top bag in the pile which had become badly infested with moths. A bottom bag, which was not infested, was tested at the same time and the strength found to be 8 parts for the retained, and 9 parts for the portion passed by the screen.

During this same period other lots of casein were stored in another building which was not heated, even in winter, and which was fairly cool in summer. These lots showed no infestation and no decrease in strength.

Other lots were stored in warehouses on "Black Tom Island" in New York between July, 1911, and August, 1913. In June, 1913, they had lost very little of their original strength and showed very little infestation with worms, but by August, 1913, most of the lots were badly infested.

This evidence seems to be conclusive as to the damage done by insects and the time of the year in which it occurs. It should also be noted that all observations were made in New York or points further north and that in warmer climates the damage would be still greater.

Fumigation.

It is generally inadvisable to fumigate packaged casein unless there is a real necessity for doing so. It is, however, necessary to check immediately an insect infestation when it first sets in. In cold climates infestation by moths may be checked by placing casein out-doors in freezing weather. If checked in this way, the bags should be swept off, placed under cover and the contents sifted and transferred to new bags if infested to any considerable extent.

In most instances of insect infestation, it is necessary to employ a fumigant such as hydrocyanic acid or sulfur dioxid. On account of the toxic dangers in the use of hydrocyanic acid for fumigation, except under exceedingly careful control, it is expedient to use sulfur dioxid.

To check the infestation of casein in bins or broken packages, the material may be sprayed with carbon tetrachloride. This treatment is especially effective for treatment of casein which has become infested by beetles of the larger sort such as Dermestes vulpinus. When such a spray is used the insects will crawl to the top and then the casein may be covered by clean burlap saturated with a highly diluted solution of hycol. This double treatment will kill insects and permit of their easy removal.

Use of Sulfur Dioxide.

When using sulfur dioxide fumigation, doors and windows must be sealed by cloth strips or otherwise, before the sulfur dioxide gas is set off

in the store room. The size of the room is first estimated in cubic feet capacity. One pound of gas per 100 cu. ft. is sufficient to destroy moths and larvæ, provided the room is left undisturbed for not less than 18 hours. Where a cylinder of sulfur dioxide is used the valve may be opened so as to allow gas to flow freely but it is best not to open the valve wide. Either a partially empty large cylinder or a small cylinder may suffice for the size of the room.

Following the treatment of stored casein with sulfur dioxide, the storeroom must be left open and thoroughly aired. Under these conditions sulfur dioxide will not permanently injure the casein in any way. It may, however, be necessary to brush off the bags, or even to sift the product and repackage in fresh burlap bags to clean it thoroughly.

The method followed at casein manufacturing plants of simply sifting it through a coarse mesh to remove webs, and fine mesh sieve to remove powder, will not permanently eliminate danger of reinfestations unless a process of fumigation is also resorted to.

Use of Sodium Cresylate.

Sodium cresylate is an excellent material for spraying bags of casein where it is necessary to resort to the use of liquid germicides. The sodium cresylate is made up as follows:

1 qt. U. S. P. Cresol.
1 qt. 50 per cent Caustic Soda.
1 qt. Water.

It does not have the objectionable odor of phenol, cresol, formaldehyde or of many articles found on the market. One part of the base is diluted with 50 parts of water. It can be used in the ordinary spray gun made for liquid plant insecticides, and used freely and with safety for spraying the exterior of the bags.

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Chapter 12

Testing and Analysis of Caseins

By E. Sutermeister

Commercial caseins differ widely in important characteristics. Not only does the nature of the acid or enzyme used to curdle the milk affect the properties, but numerous other details of the manufacturing process such as the temperature of precipitation, the washing of the curd, whether or not the curd is "cooked," how long and under what conditions the curd remains in the wet condition before drying, the temperature of drying, the fineness of grinding, the length of time and conditions of storage of the dry casein, all may affect its behavior to an important degree. The resulting variations in the product are great enough to make it necessary in some of the important applications to alter formulas to suit the requirements of different shipments of casein or else to restrict purchases to a limited number of casein makers known to be selling the kind of casein desired. Moreover, the kind of casein generally preferred in one industry may be regarded as quite inferior for some other line of work.

In spite of the urgent need of practicable methods for evaluating commercial caseins for use in the several industries, no really adequate systems of testing are available at the present time. Several empirical tests of a physico-chemical character have been proposed, such as the "borax solubility," the "viscosity," the "strength," and the "acidity" tests, but much yet remains to be done in determining precisely the significance of these tests for the various applications of casein. A system of proximate chemical analysis [1] was used by the Government during the War as the basis for the purchase of casein for glue making. It was adopted in an emergency calling for prompt action and affording no time for the development of more refined methods. Although it has been criticised severely [2] and is admittedly cumbersome and inadequate, no one has yet given the subject enough study to work out a method having greater significance for the use of casein in glue.

A somewhat similar condition exists in the paper coating industry. Here the strictly chemical tests of casein are impossible of interpretation at the present time and judgment is passed on the basis of the physico-

chemical tests already mentioned. While these are quite satisfactory as a means of grading caseins at any one plant they are of little value in establishing specifications because they entail the use of standardized materials, and also because special training is necessary if different workers are to carry them out in the same way.

In the present state of our knowledge of the subject, such a combination of the tests described in this chapter as prove practicable for the particular case in question may be used to make sure that different shipments are uniform in general character, and are of a reasonably good quality. Whenever possible, however, they should be supplemented by small scale tests of a sample of each shipment in the formula in which it is to be employed.

Cleanliness.

The best way to judge the cleanliness of casein is by examination under the microscope at low magnification, or by the use of a good hand lens. In the absence of such facilities it may be subjected to careful visual inspection of the material as received. A third method is to make a moderately dilute solution, say 25 grams of casein in 500 cc., allow it to stand for a short time, and note the dirt which settles out or floats on the surface. When examined in this way an idea can frequently be gained of the nature of the dirt and sometimes of its source.

Absence of dirt, or at least the presence of a minimum amount, is important in most of the industries in which casein is used. The most notable exception to this is glue making in which several per cent of dirt might not cause enough trouble to injure the glue seriously. There are no fixed standards for cleanliness to which casein must conform, and the amount of dirt permissible must be judged from experience, or by comparison of the sample with standards of acceptable quality. Obviously the methods described are not sufficiently precise to incorporate in a specification for purchase unless a standard can be agreed upon between buyer and seller.

Color.

In the case of color also there are no generally accepted standards for casein and no scientific method for its determination. Probably the best procedure is to compare the specimen with standard samples. Even this is not entirely satisfactory because the color of casein changes with time, especially if it is exposed to light, and the yellow color becomes much less noticeable the more finely the sample is ground. Casein of good quality need not be darker than a pale yellow or cream color, and for use in glue making even a distinct reddish color may not indicate unsatisfactory quality.

Odor.

Browne[1] suggests the following test: "About 10 grams of the casein are soaked in about 10 cc. of water and an equal volume of rather thick 'milk of lime' added with stirring. After the mixture has stood a few minutes the odor is noted." High grade commercial caseins should have very little odor, or at most, an odor resembling that of sweet milk. A disagreeable odor may not always indicate inferior quality, for the casein may have acquired odors during transit from other materials stored near it. The rancid odor, which is frequently considered characteristic of casein is not due to the casein itself, but to associated impurities or decomposition products.

Fineness.

Casein may be graded for fineness by screening on standard sieves. They should be shaken in standard manner and for a definite length of time. Browne[1] suggests placing a 50-gram sample on a 60-mesh screen and shaking by hand in a horizontal plane at a rate of about 120 strokes per minute. At the end of each stroke the sieve is allowed to strike the palm of the other hand which is held stationary. At the end of ten minutes the portion passing through is weighed.

The test for fineness in casein is particularly important in grading it for use in lime-casein spreaders, in cold water paints and for glue making. It may also be employed to advantage in the coated paper industry where the use of too finely ground casein is objectionable.

Solubility.

One of the earliest solubility tests to be generally applied to casein consisted in treating 50 grams of casein, ground to pass a 20-mesh sieve, with 300 cc. of water containing 7.5 grams of borax. When stirred continuously at a temperature of 65° C. solution should be complete in 10 minutes. This test was largely employed to determine the suitability of the casein for coating paper, but it did not tell the whole story, and its results needed to be interpreted with care, for many caseins which were not completely soluble by this test gave satisfactory results in use.

A test similar to this can be worked out in any industry, or any particular plant, to suit its own special requirements for solvents. Under such conditions it may give valuable indications of the suitability of the casein for the work in question. This is especially true if the character of any insoluble residue is taken into consideration.

An improved borax solubility test has been described by Zoller.[3] Fifteen grams of casein, ground to pass a 40-mesh sieve, are weighed into a 250 cc. beaker and 100 cc. of a 0.2 M borax solution (76.32 grams $Na_2B_4O_7.10H_2O$ in 1 liter) at a temperature of 30° C. added with vigor-

ous stirring. Allow to stand for 30 minutes, with thorough stirring at intervals of 5 minutes, and frequent stirring during the first 5 minutes. Until familiar with the test use a casein of known qualities for comparison.

This test does not show the suitability of the casein for any commercial work, but it does differentiate between caseins made at high and low temperatures. Cooked curd caseins always tend to imbibe water and form a jelly.

Viscosity.

The viscosity of casein solutions may be measured by any of the standard methods involving either the principle of time of efflux from a standard orifice or the torsion of a wire caused by the resistance of the casein solution to the rotation of a plunger. If comparatively dilute solutions are to be measured the methods using the orifice principle are probably the best, but if rather heavy solutions, or those containing suspended solids are to be tested the torsional methods are to be preferred.

There is no general standard for viscosity, to which all caseins are expected to conform, and, in fact, different industries have very different requirements. It is therefore necessary that each industry, or even each individual plant, should work out its own standard method of testing which should be so planned as to give information of value in that particular work. It should also be remembered that the viscosity of a casein solution varies with the kind of solvent used, with the fineness of grinding of the casein, and in some cases at least, with numerous details of time, temperature, stirring, etc., in the preparation of the solution. In glue making the viscosities of the final mixtures made with different caseins may not agree with predictions based upon the viscosities of their solutions in, let us say, borax. In the coating of paper it has not proved possible to determine the viscosity of a casein solution and from the result predict that of the coating mixture which it will make when mixed with clay and satin white. Judgment of the value of the casein must, therefore, be based on the viscosity of the final coating mixture, rather than on that of the casein solution.

It should also be pointed out that no one has yet made a study to find out which casein solutions, if any, are *viscous* and whether the more important solutions are to be regarded as *plastic* or perhaps of a still more complicated flow character requiring three or more parameters for its description.

Strength.

The term "strength" as applied to casein means different things according to the various industries in which it is used. In glue work it is used in the sense of cohesive power or tensile strength, and it is generally

thought of as a property of the mixture rather than of the casein. The tests applied in the woodworking industry, as described in the chapter on "Glues," are in reality chiefly tests of the method of gluing up the specimen, and can be considered as only partly indicating the strength of the glue, and not at all that of the casein.

Any test to show the strength of casein should approximate the conditions under which it is actually used. Such a test, which has proved very satisfactory to the makers of coated papers, is as follows:[4] One hundred grams of clay, previously dried at 100° C., are weighed into a heavy walled, porcelain cup and soaked up with 70 cc. of water. A casein solution is prepared with any desired solvent and is so adjusted, by adding water if necessary, that each 5 grams of the solution contains just 1 gram of the casein to be tested. The clay and water are mixed until free from lumps, which may be done conveniently with a copper spatula, bent nearly at right angles on the end to form a foot with which to break up any masses of clay that are not easily reduced. The cup and its contents, including the spatula, are balanced on the scales and 30 grams of the casein solution are weighed in and stirred until a homogeneous mixture is obtained. A little of this coating mixture is transferred to a small piece of paper and spread to a thin, uniform layer, either by means of a small, flat brush or by means of a scraper. The sheet is marked "6 grams of casein" and is then laid aside to dry. The cup is replaced on the scales, 5 grams more of the casein solution added, and another sheet spread as before and marked "7 grams." This is repeated until casein solution equivalent to 12 grams of casein has been used. A scraper for spreading the coating mixture may be made from a piece of saw blade about 4 inches wide and 5 inches long, bent into a rather flat arc and ground smooth on the convex side at both ends. When this is held with the convex side down and drawn across the paper a very even coating may be applied, after sufficient skill has been obtained by practice.

After the sheets of coated paper are thoroughly dry each one is held up to the light and several small areas having the desired thickness of coating are marked out. Short pieces of high-grade sealing wax are then heated at one end until nearly melted and one applied to each of the marked areas. When they have cooled a finger is placed on either side of the wax to hold the paper firmly, and the wax is removed by a steady, vertical pull. If enough casein is present the surface of the wax will be covered with fibers to the very edge, if too little was used the coating comes away from the paper without pulling off any fiber, while there is usually an intermediate case in which the center of the wax shows fiber and the edges clay only; this would be considered just on the line between weak and strong.

This is an excellent comparative test but if reliable results are to be

obtained the same clay and paper must be used and places in which the coating is of the same thickness must be selected for applying the wax. As the influence of all these factors is very appreciable the results obtained in different laboratories would not be comparable unless the same materials could be distributed to all, and the operators trained to produce coatings of a standard thickness.

Moisture.

According to Shaw [2] the moisture in casein should be determined by drying a 3-gram sample for 5 hours at 98° C. in a partial vacuum. A large double-walled water oven will give fairly good results if the samples are placed in the center, but an electrically heated oven may not be satisfactory if there are considerable variations in temperature at different points. Weighings should be made in stoppered weighing bottles as open dishes have been found to give low results. Browne [1] found that drying in an evaporating dish in a Freas oven at 98° for 5 hours at atmospheric pressure gave a maximum of 0.47 per cent less than drying in vacuo.

Casein is a strongly hygroscopic material, parting with the last traces of moisture reluctantly and reabsorbing moisture very readily. In dealing with any material of this kind, the oven employed, as well as the technic for handling the dried samples, should be subjected to careful study to make sure that reproducible results are being obtained. Probably the true moisture content cannot be obtained by any practicable method.

Ash.

Weigh the sample into a silica dish and heat over a very low flame, taking care that the contents of the dish do not burst into flame. It should require about an hour to char the casein thoroughly, after which the dish is placed in a muffle furnace and the ignition completed at as low a temperature as is consistent with obtaining a white, or nearly white, ash. Shaw [2] finds this procedure satisfactory with caseins of medium or high ash content, but with low-ash casein there is not enough base present to hold back the organic phosphorus and low results are a consequence. This loss may be avoided by moistening the casein with 5 cc. of calcium acetate solution containing a known amount of calcium oxide, drying in an oven and then igniting as described. From the ash thus obtained deduct the CaO added as acetate and consider the balance as coming from the casein. Table I shows the effect of this treatment on the ash constituents of several caseins.

There is a decided difference of opinion as to which of these methods is the more satisfactory for low-ash caseins, but whichever side of the argument is supported it should be remembered that the ash determination, as ordinarily made, is very useful to the glue maker and enables him

TABLE I

EFFECT OF ADDED CALCIUM ACETATE ON THE DETERMINATION OF ASH CONSTITUENTS [2]

Method	Low Ash Casein	Medium Ash Casein	Medium Ash Casein	High Ash Casein
	per cent	per cent	per cent	per cent
Ash, untreated sample, porcelain dish	1.51	2.41	3.09	4.13
Ash, untreated sample, quartz dish	1.68	2.45	3.22	4.20
Ash, 5 cc. $Ca(C_2H_3O_2)_2$ sol. (0.1523 g. CaO added)	2.25	2.66	3.23	4.60
Ash, 5 cc. $Ca(C_2H_3O_2)_2$ sol. (0.0761 g. CaO added)	2.23	2.54	3.26	4.52
P, untreated sample	0.51	0.77	0.74	1.05
P, 5 cc. $Ca(C_2H_3O_2)_2$ sol. (0.1523 g. CaO added)	0.80	0.80	0.88	1.04
P, 5 cc. $Ca(C_2H_3O_2)_2$ sol. (0.0761 g. CaO added)	0.80	0.80	0.89	1.04

to gage the amount of water to use with his casein. If Shaw's modification is adopted the information obtained is no longer of any value in telling how the casein should be used in glue making, at least until some one repeats the routine work of determining ash contents and making glues from a large number of caseins. If enough study could be given to the subject it would probably be found that the knowledge desired by the glue maker is the calcium oxide content of the casein and not the ash.

Fat.

The method recommended by Shaw [2] is a modified Roese-Gottlieb method. Weigh accurately about one gram of the casein into the Roehrig tube, and add 10 cc. of water. Shake vigorously, but not so as to carry particles of the casein near the top of the tube, and allow the casein to soak for at least 15 minutes. Add 2 cc. of strong ammonia water, and shake vigorously, again taking care not to carry particles of casein near the top of the tube. Let the tube stand 10 minutes with occasional shaking, then add 10 cc. of 95 per cent alcohol and shake until the casein is completely dissolved. Add 25 cc. of ether, shake thoroughly for half a minute, add 25 cc. of petroleum ether and shake again for half a minute. Allow to stand for about 20 minutes, or until the liquids have separated, and draw off the ether solution as completely as possible, filtering through a pledget of cotton into a weighed flask. Repeat the extraction with ether and petroleum ether, using 15 cc. of each solvent. Evaporate the combined extracts and dry the residue at 100° C. to constant weight.

This method gives higher results than the continuous ether extraction as applied to the solid casein. There is good evidence that the latter

method does not remove all the fat, unless the casein is ground to an exceedingly fine powder. In confirmation of this Shaw [2] determined the fat in a low-ash casein and found 0.08 per cent by the ether extraction method as compared with 1.50 per cent by the modified Roese-Gottlieb method.

TABLE II

COMPARATIVE FIGURES FOR FAT OBTAINED BY THE ETHER-EXTRACTION METHOD AND THE MODIFIED ROESE-GOTTLIEB METHOD [3]

Sample	Fat by the Ether-Extr. Method, per cent	Fat by the Modified Roese-Gottlieb Method, per cent
Grain-curd casein	0.08	1.50
H_2SO_4 cooked-curd casein...	0.20	2.35
Buttermilk casein	3.79	3.92

Another method for fat which has been found very convenient, but which has not been checked against the modified Roese-Gottlieb method for accuracy, makes use of the Babcock milk tester.[5] Soak 2 grams of casein in 6 cc. of water in a small beaker and after about half an hour add, with constant stirring, 9 cc. of concentrated sulfuric acid (sp. gr. 1.84). Pour the solution into the Babcock skim-milk bottle and wash out the beaker with 5 cc. of water and 5 cc. of sulfuric acid. Fill to the base of the neck with dilute sulfuric acid (4 cc. water and 5 cc. concentrated acid), and whirl in the centrifuge for 5 minutes. Fill with dilute acid, whirl 2 minutes and while still hot read the fat on the graduated neck. The reading multiplied by nine gives the per cent of fat in the casein. The secret of success with this method is in getting the concentration of the acid just right; if it is too strong it will char the fat while if it is too weak the casein will not all be in solution and a reading will be impossible. This method also gives much higher results than the ether extraction of the solid casein.

Sugar.

Weigh 10 grams of the casein into a 500 cc. wide-mouthed bottle and add 250 cc. of 50 per cent alcohol. Shake 4 hours in a motor-driven shaking machine, allow to stand for several hours, and decant the solution. Centrifuge the solution if it is turbid and take 10 cc. of the clear solution for the sugar determination, which is carried out according to the method of Bryan, Given and Straughn. The per cent of sugar in various caseins is given in Table III.

"Acidity."

The method for "acidity" given by Browne [1] is as follows: A 1-gram sample is placed in a flask and 25 cc. of 0.1 *N* sodium hydroxide solution

TABLE III.

SHOWING PER CENT OF SUGAR IN SAMPLES ANALYZED [3]

Sample	Sugar Per cent
Natural-sour casein	Trace
H_2SO_4 cooked-curd casein	1.13
Grain-curd buttermilk casein	0.95
H_2SO_4 cooked-curd buttermilk casein	0.62
H_2SO_4 uncooked-curd buttermilk casein	0.33
Grain-curd casein, not washed	3.96
Grain-curd casein, washed well	1.43
Grain-curd casein from partly sour skim milk, not washed	5.43
Grain-curd casein from partly sour skim milk, washed well	0.56

run in from a pipette. During this addition the flask is gently agitated. The flask is then stoppered and agitation continued until the solution is complete. The stopper is then removed and the portion of solution wetting it is washed into the flask with a stream of water from a wash bottle; 100 cc. of distilled water (neutral to phenolphthalein) are added, and the solution back titrated at once with 0.1 *N* acid, using 0.5 cc. of alcoholic phenolphthalein solution (1 gram per 100 cc.) as indicator. The acid is run in fairly rapidly with vigorous shaking of the flask so as to prevent precipitation of the curd locally. The number of cubic centimeters of 0.1 *N* alkali used up by 1 gram of moisture-, fat-, and ash-free casein is called the "acidity" of the sample.

Pure isoelectric casein under these conditions is said to combine with about 9.0 cc. of 0.1 *N* alkali. Commercial casein may have either higher or lower "acidity" depending on the presence of free acid, proteins other than casein, or decomposition products on the one hand, or lime, etc., on the other. There is a close relationship between "acidity" and ash content; other things being equal, the higher the ash content the lower the "acidity."

If this method is to give concordant results several precautions must be observed. The flask must be kept stoppered except when making additions or titrating. The amount of indicator must not vary from that specified and it must be adjusted with alkali so that one drop added to distilled water does not change its reaction. Local coagulation of casein during the titration must be guarded against, and the total time the casein is in contact with the alkali must not exceed 30 minutes at room temperature.

A much more refined procedure where the equipment is available is to titrate electrometrically, plotting a "buffer" curve, instead of titrating to an arbitrary pH.

Phosphorus.

For medium-, or high-ash caseins dissolve the ash in 5 to 10 cc. of concentrated nitric acid on the steam bath; wash into a small beaker and

boil to oxidize the phosphorus; neutralize with ammonia and then make faintly acid with nitric acid. Add 2 grams of ammonium nitrate; heat nearly to boiling and add an excess of ammonium molybdate solution. Digest for an hour at about 65° C. and test the clear, supernatant liquid with more molybdate solution to make sure that precipitation is complete. Filter and wash with water or ammonium nitrate solution, and then dissolve the precipitate from the filter with hot ammonium hydroxide. Wash the filter with hot water, collect both the solution and washings in a small beaker until the volume does not exceed 100 cc. Nearly neutralize with hydrochloric acid, cool and add slowly, and with constant stirring, an excess of magnesia mixture. After about 15 minutes add 12 cc. of ammonium hydroxide (sp. gr. 0.90). Let stand for at least 2 hours, and preferably over night, filter, wash with dilute ammonium hydroxide until practically free from chlorides, dry, ignite until white or grayish white, and weigh as $Mg_2P_2O_7$.

As explained in connection with the ash determination, unless more base is added some of the organic phosphorus is lost in ashing a low-ash casein. For such caseins the phosphorus should be determined on the ash obtained after treating the casein with calcium acetate solution, or the casein may be treated with magnesium nitrate solution, ignited and the ash dissolved in hydrochloric acid according to the method for total phosphorus in fertilizers.[6] In case hydrochloric acid is used as a solvent add 15 grams of dry ammonium nitrate to the solution before precipitating with ammonium molybdate.

Calcium.

Weigh accurately 1 to 2 grams of casein, and heat in a crucible until the material is charred. Digest the residue with dilute hydrochloric acid; filter; dry the filter; ignite it and repeat the extraction with hydrochloric acid. Unite the acid extracts and make up the solution to 100 cc. To 50 cc. of the solution add 1 cc. of concentrated sulfuric acid and heat to boiling. Add 100 cc. of alcohol, stir well and allow to stand over night. Collect the precipitate in a weighed Gooch crucible, wash it with alcohol until free from acid, dry it at 120° C. and weigh as calcium sulfate, $CaSO_4$. The calcium may also be determined by digesting the casein with aqua regia and then following the customary procedure of precipitating with ammonium oxalate and igniting to calcium oxide.

Nitrogen.

This is best determined by the usual Kjeldahl method. According to Browne [1] the per cent of nitrogen found multiplied by 6.38 will give the per cent of pure casein in the sample, while Höpfner and Burmeister [7] state that nitrogen times 6.61 gives fat-, ash-, and moisture-free material.

From the analyses of the latter authors it appears that the factor 7.70, applied to the nitrogen per cent, will give commercial casein containing the average amounts of fat, ash, and moisture. Analyses given by Bogue,[8] exclusive of the results for buttermilk and rennet caseins, indicate a factor of 7.73, which is in very close agreement with that of Höpfner and Burmeister. This factor, 7.70, is useful in calculating the amount of commercial casein where it has been used as an adhesive in such materials as coated papers, etc.

Miscellaneous.

The micro-organism count and the Warth incubation test will be found described in full in the chapter on "Storage." These tests are of value in determining the conditions of cleanliness during the manufacture of the casein, and in selecting caseins which will stand up well during storage.

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